STRATEGIES FOR DENGUE EARLY WARNING, SURVEILLANCE AND CONTROL

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

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Declaration

This work has not been previously accepted in substance for any other degree and is not being currently submitted in candidature for any other degree.

Signed .... (Candidate)

Date .... 12th November 2015 ....

This thesis is the result of my own investigations. The results presented in chapters 2,3, 4 and 5 were obtained in collaboration with others, whose contributions were as follows:

Chapter 2

LRB and PJM conceived and designed the experiments. LRB analysed the data. LRB wrote the manuscript with edits and comments from PJM and SRR[c]. LRB, SRR and PJM contributed to the review protocol. LRB carried out literature searches. LRB and PJM reviewed the literature.

Chapter 3

LRB conducted the searches. LRB and PJM extracted the data. LRB analysed the data with guidance from SD[d]. LRB wrote the manuscript with comments and edits from PJM and SD.

Chapter 4

PJM designed the trial with input from LRB. LRB managed the trial overseas with remote support from PJM. LRB wrote the manuscript with comments and edits from PJM.

Chapter 5

AK[e] and PO[f] designed the study, with input from LRB, SRR, PJM and MP[g]. LRB, GT[h], BSG[i], GC[j], LH[k], SRR, LCQ[l] and RESR[m] collected the data. MP wrote code for the statistical analyses. LRB conducted all analyses presented in the manuscript while MP provided comments and guidance. LRB wrote the manuscript with comments and edits from MP PJM, BSG, AK and SRR.

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Dedicated to my grandfather William 'Bill' Hambling.

Abstract

Dengue is a neglected tropical disease of global importance today. Transmitted by the mosquito vectors *Aedes aegypti* and *Aedes albopictus*, dengue afflicts both urban and rural human populations in a cycle of endemic and epidemic transmission. As the global dengue burden continues to grow, there is an urgent need for timely and effective vector control, the only means to prevent transmission of dengue. This thesis addressed these contemporary dengue challenges by investigating three key elements of dengue outbreak alert and response.

At present, entomological surveillance protocols are used to quantify vector abundance as a measure of dengue transmission risk, and although routinely undertaken in numerous endemic areas, there has been no evidence-based consideration of their reliability or accuracy. Similarly, vector control tools and approaches are numerous and widely used, especially during outbreaks, despite insufficient evidence of effectiveness and impact on dengue transmission. Finally, effective early warning systems could provide sufficient time to mobilise resources for a timely response to possibly mitigate the impact of dengue outbreaks.

A systematic review of the literature explored the evidence for the value of entomological indices and dengue transmission. Of 13 studies investigating associations between vector indices (mainly the Stegomyia indices) and dengue cases, 4 reported positive correlations, 4 found no correlation and 5 reported ambiguous or inconclusive associations. Single values of the Breteau Index (BI), widely used as dengue transmission thresholds, were shown to be unreliable. Hence, there is little evidence that vector indices correlate with dengue transmission, although some methods, such as adult mosquito indices, merit further research.

The effectiveness of vector control tools was examined in a systematic review and meta-analysis. Of 41 studies eligible for inclusion, 19 provided sufficient data for meta-analyses. Though evidence was weak, reduced odds of dengue incidence were observed for house screening from 3 trials (Pooled OR: 0.22 (95% CI 0.05, 0.93)). 3 community-based combination interventions significantly impacted mosquito indices: BI Rate Ratio (RR) 0.48 (95% CI 0.26, 0.89); BI RR 0.65 (95% CI 0.52, 0.81); BI Mean difference (MD) -4.66 (-5.89, -3.43). Remarkably, impact on dengue cases by fogging, a method widely used during outbreaks, had never been evaluated in randomised trials; only one study demonstrated effectiveness against the vector.

Effectiveness of vector control methods were also analysed in a 1-year randomised controlled trial, in particular, indoor/ outdoor fogging, indoor residual spraying and handheld spray cans.

Finally, a retrospective study of data from 5 countries in Asia and Latin America was conducted to prospect for alarm signals that potentially could warn of impending dengue outbreaks. The Shewhart method and Endemic Channel identified probable dengue cases and mean temperature as predictors of outbreaks, with sensitivities and positive predictive values of 95% and 48% in Dominican Republic, 86% and 44% in Mexico, indicating that these predictors could be beneficial if utilised in early warnings systems.

This thesis has highlighted fundamental knowledge gaps in dengue transmission dynamics and vector control that are crucial for effective outbreak warning and response systems. These must be addressed before existing or novel vector control tools can be optimised, with or without an efficacious vaccine, to reduce endemic and epidemic dengue.

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LIST OF ABBREVIATIONS

BI .... Breteau Index
CDC .... Centres for Disease Control
CHIKV .... Chikungunya Virus
CI .... Confidence Interval
CI .... Container Index
CRCT .... Cluster-Randomised Controlled Trial
CWG .... Community Working Group
CWG .... Community Working Group
DENV1 - 4 .... Dengue Virus Serotypes 1,2,3,4
DENV .... Dengue Virus
DIY .... Do-It-Yourself
EIP .... Extrinsic Incubation Period
ENSO .... El Nino Southern Oscillation
EU .... European Union
GPS .... Global Positioning System
HI .... House Index
ICC .... Intra-Cluster Correlation
IRS .... Indoor Residual Spraying
ITC .... Insecticide Treated Curtain
ITM .... Insecticide Treated Material
ITWC .... Insecticide Treated Water Cover
IVM .... Integrated Vector Management
KAP .... Knowledge, Attitudes, Practices
MD .... Mean Difference
MMR .... Measles, Mumps, Rubella
NGO .... Non-Governmental Organisation
NS1 .... Non-Structural Protein 1
OR .... Odds Ratio
PCR .... Polymerase Chain Reaction
PPI .... Pupae Productivity Index
PPV .... Positive Predictive Value
QATQS .... Quality Assessment Tool for Quantitative Studies
RCT .... Randomised Controlled Trial
RR .... Rate Ratio
WHO .... World Health Organisation
YF .... Yellow Fever

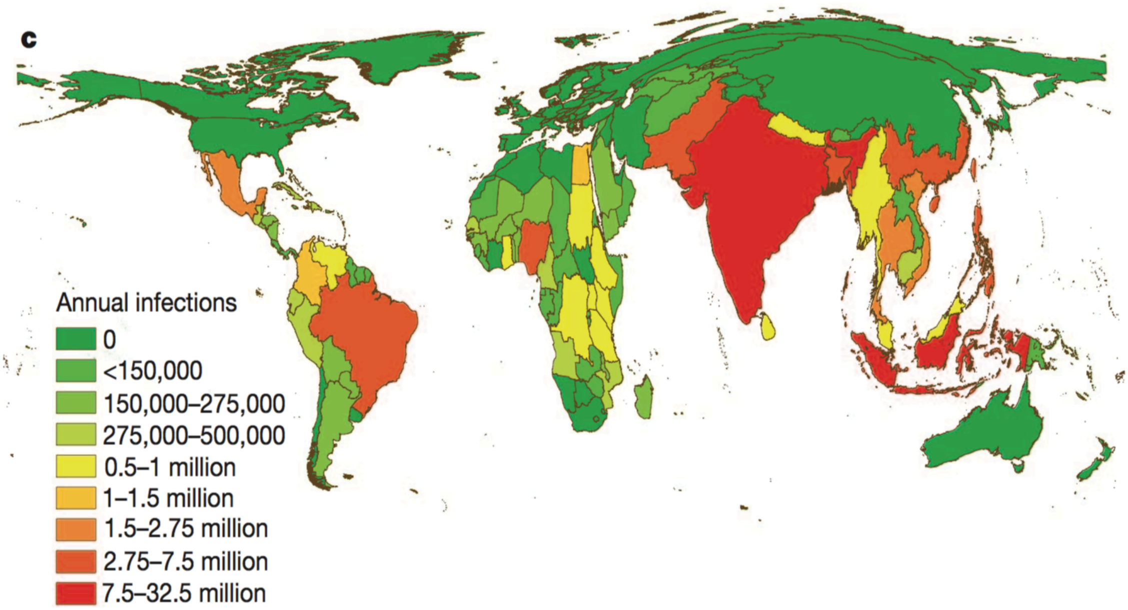
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Dengue

Dengue is believed to have originated as a mammalian disease in non-human primates and emerged in the human population roughly 500-1,000 years ago (Wang et al. 2000). It is estimated to infect 390 million people annually (Figure 1.1) (World Health Organisation 2012a; Bhatt et al. 2013) and remains endemic in a multitude of countries. Indeed, almost half the world's population remain exposed to infection, with the Asia-Pacific region accounting for 75% of the worldwide dengue burden (World Health Organisation 2012a). While incident metrics and population-at-risk estimates abound, the economic cost of frequent dengue outbreaks remains poorly understood (Stahl et al. 2013).

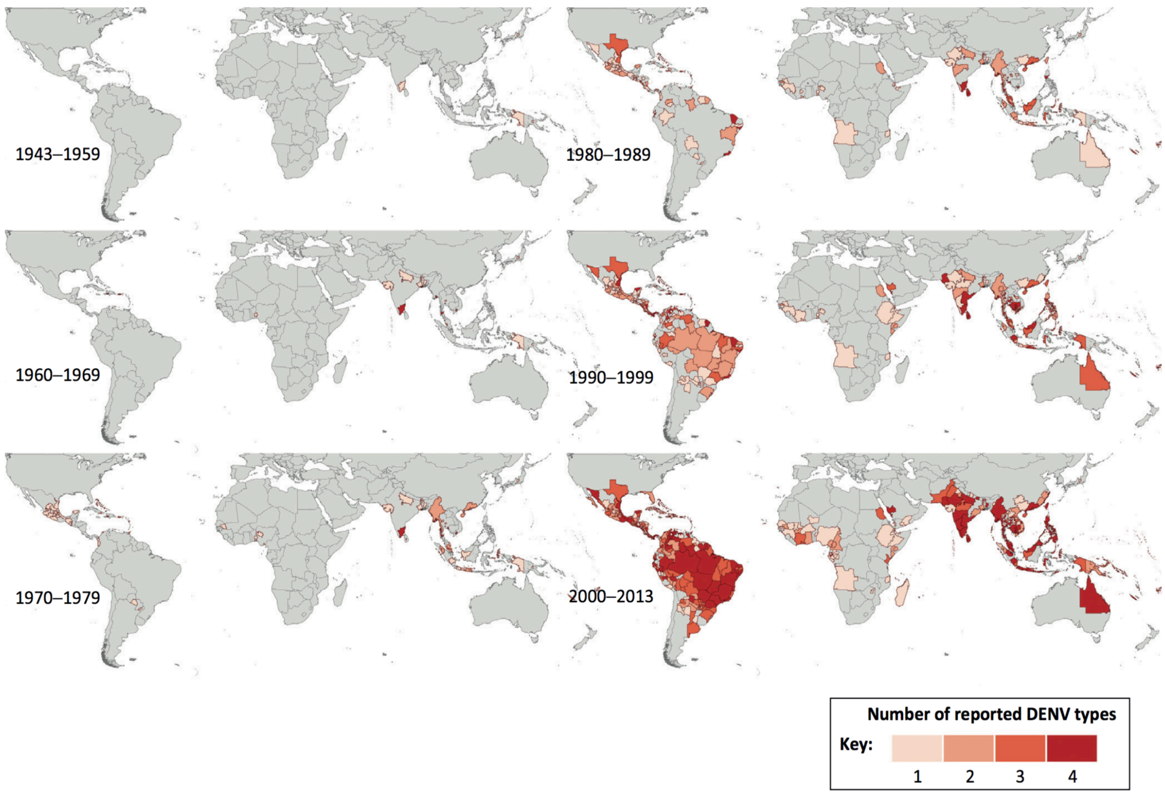
Figure 1.1. Cartogram of the annual number of infections for all ages (colour) as a proportion of national or subnational (China) geographical area (size) (Bhatt et al. 2013).



Dengue is caused by a flavivirus of 4 virus serotypes (DENV1, DENV2, DENV3, DENV4) that share approximately 65% genetic homology, which can vary between isolates

(Guzman et al. 2010; Katzelnick et al. 2015). Over the past 20 years, these serotypes have spread worldwide from South East Asia and are now found throughout Asia, Africa and the Americas (Figure 1.2) (Guzman et al. 2010; Messina et al. 2014). International travel, trade, migration, restricted access to health care and urbanisation are considered among the main drivers behind the rapid dissemination of all four dengue serotypes (Weaver 2013; Messina et al. 2014; San Martin et al. 2010). Compounding the problem has been the global spread of the major dengue mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, throughout the last century (World Health Organisation 2012a; Weaver & Reisen 2010).

Figure 1.2. DENV Co-circulation. Cumulative number of DENV types reported by decade since 1943 in Messina *et al., 2014* (Messina et al. 2014)



Dengue Vectors

Aedes aegypti

Dengue is transmitted primarily by the female mosquito *Aedes aegypti* (Simmons et al. 2012), which thrives in and around urbanised areas. It is diurnal and highly anthropophilic, with domestic forms showing increased propensity towards exclusive human feeding (McBride et al. 2014). It has greater competency for transmission than *Ae. albopictus* (Lambrechts et al. 2010), and coupled with short, frequent biting behaviour, it can transmit dengue multiple times during a single gonotrophic cycle (World Health Organisation 2012a; Scott & Morrison 2010). It bites during the day (Gubler & Clark 1995), attracted to human odorous compounds such as CO_2, lactic acid, sulphides and ketones (Paixão et al. 2015; Bernier et al. 2015). The strength of this attraction is dependent on the mating status of the female, as research has shown that mated mosquitoes are more attracted to human odour than unmated mosquitoes (Paixão et al. 2015). Once a host is detected, visual cues, such as darker colours and movement, confirm the presence of a viable host immediately prior to landing (Kennedy 1940). Feeding occurs after an initial probing of the skin surface (Clements 1999), and once engorged, the mosquito prefers to rest indoors to begin the gonotrophic cycle (Clements 1999). Subsequently, the female mosquito preferably seeks out large containers of freshwater (Harrington et al. 2008), although most container types in and around the home are suitable (World Health Organisation 2009; World Health Organisation 2012a). Upon reaching a container, oviposition occurs, where the female lays eggs singly just above the water's edge (Fay & Eliason 1966), even though some eggs may be found in the water (Abreu et al. 2015). Unique to *Aedes*, the mosquito will deposit portions of her 100-200 egg batch in multiple breeding sites, perhaps as many as 11, if available (Abreu et al. 2015; Williams et al. 2008) - a process known as 'skip oviposition'. These eggs are particularly resistant to desiccation for prolonged periods (World Health Organisation 2009) and considering this, it is not surprising that the geographical range of *Ae. aegypti* has increased over the last century, with oceanic trade contributing to the spread of *Aedes* eggs, as well as increased prevalence of mosquito microhabitats due to urbanisation of the tropics (Weaver & Reisen 2010). This ability to withstand relatively extreme environmental and climatic variation has resulted in detection of the mosquito up to the spatial boundaries defined by the 10°C winter isotherms (latitudes of 35°N/ 35°S) (World Health Organisation 2009).

Aedes albopictus

The secondary vector, *Aedes albopictus*, is also diurnal but less dependent on humans for blood meals (Ngoagouni 2015). Indeed it is zoophilic and will feed on warm- and cold-blooded species, but preferentially feeds on humans (Ngoagouni 2015). Based on these observations, the implication is that this species is the most likely bridge vector between non-human primates and human populations (Smith 1956), although other competent vectors, such as *Ae. polynesiensis* and *Ae. scutellaris* may also be responsible (Vasilakis 2011). In times past it was considered a rural mosquito, often breeding in tree holes and bromeliads (Higa 2011). However it has emerged as a highly adaptive mosquito that can now successfully breed in man-made containers, including tyres and household receptacles (Li et al. 2014), thereby increasing its potential as a serious vector of dengue (Brady et al. 2014; Li et al. 2014; World Health Organisation 2012a). Termed the "Asian Tiger Mosquito", its range has expanded dramatically over the last 30 years, establishing in the Americas, Australia, Africa and Europe (Lambrechts et al. 2010), in part due to the used tyre trade, 'lucky bamboo' and increasing urbanisation (World Health Organisation 2012a). Accordingly, it is currently expanding the boundaries of known dengue transmission (Eurosurveillance 2013; Añez & Rios 2013). Yet, it appears that its heterogeneous biting behaviour reduces the vectorial capacity of this vector, as it is not principally responsible for large dengue outbreaks (Brady et al. 2014; Lambrechts et al. 2010). Indeed, across the islands of Guam, Taiwan and Hawaii, wherein human populations had low prevailing herd immunity to all four dengue serotypes, an abundance of *Ae. albopictus* did not further epidemic dengue transmission (Lambrechts et al. 2010). Nevertheless, *Ae. albopictus* is considered an important bridging vector of arboviruses (Lambrechts et al. 2010) and its presence may be a crucial factor to dengue endemicity among otherwise naïve human populations. Sporadic incidence of similar arboviral diseases such as Chikungunya, also highlights the geographical range of these *Aedes* species, and how dengue incidence may establish among susceptible populations, particularly throughout Eurasia (Beltrame et al. 2007; Rezza et al. 2007).

Aedes Genera

Additional *Stegomyia* mosquitoes incriminated as competent vectors of dengue at local spatial levels include *Ae. henselli, Ae. furcifer* and *Ae. luteocephalus*, yet due to their limited geographical range, they are considered of secondary importance in dengue transmission (World Health Organisation 2012a).

Dengue Transmission Dynamics

All four virus serotypes are transmitted by female *Aedes* vectors horizontally between humans, with an extremely small proportion transmitted vertically to mosquito eggs (World Health Organisation 2009). On rare occasions, blood products (Oh et al. 2015), organ transplantation (F. L.-S. Tan et al. 2005) and nosocomial needle-stick injury can result in dengue transmission (Morgan et al. 2015). However, the vast majority of dengue cases result from the bite of an infected female *Ae. aegypti* or *Ae. albopictus*.

Extrinsic Incubation Period

The extrinsic incubation period (EIP) is best described as the time necessary for a newly infected mosquito to incubate the virus ready for subsequent transmission (Chan & Johansson 2012). During feeding, the female mosquito ingests the virus with the blood meal from an infected human host (Scott & Morrison 2010), marking the beginning of the EIP. Thereafter, virus particles enter the midgut and systematically migrate to all tissues, including the salivary glands, from which point transmission can occur during subsequent bites (World Health Organisation 2009). This time point marks the end of the extrinsic incubation period, a process that has usually taken between 8-12 days (Watts et al. 1987; World Health Organisation 2009), and signals the beginning of lifelong infectiousness for the mosquito (World Health Organisation 2009). Factors affecting the EIP primarily include: 1) ambient temperature (Chan & Johansson 2012), which if elevated, can reduce the EIP to as low as 5 days (Ritchie et al. 2013); and 2) mosquito species, as dissemination rates from the midgut of *Ae. albopictus* to surrounding tissues are notably lower than in *Ae. aegypti* (Lambrechts et al. 2010).

Intrinsic Incubation Period

The intrinsic incubation period, the time from the initial infective bite necessary for a human to become symptomatic/ infectious, is typically 4-7 days, with a mean of 5.9 days and a known range of 3- 15 days (Chan & Johansson 2012; Scott & Morrison 2010). Upon entering the bloodstream, virus particles infect immature dendritic cells, which travel to the lymph nodes upon maturation (Guzman et al. 2010). Upon stimulating the immune response, macrophages and a range of other cell types become infected and circulate to locations as diverse as the liver, spleen and kidneys, where further replication likely ensues (Jessie et al. 2004; Guzman et al. 2010). Once replication is complete, mature virus particles bud from infected cells, at which point they can be taken up in subsequent mosquito blood meals (Rodenhuis-Zybert et al. 2010).

Transmission Thresholds

Dengue transmission thresholds originated during the 20th Century, when transmission metrics were formulated for another flavivirus, yellow fever (YF), also transmitted by *Aedes* vectors (World Health Organisation 1971). These were based on mosquito abundance data and used as a proxy for transmission risk. Some of the earliest evidence for the inception of such 'risk thresholds' began to emerge with yellow fever investigations in the 1920s, when Connor et al. (1923) proposed that a mosquito container index (CI) (defined pp.12) of <10% indicated a safety zone (Connor & Monroe 1923). Then, later in 1956, MacDonald et al (1956) proposed that a mosquito House Index (HI) (defined pp.12) threshold of less than 1 was indicative of low risk for YF transmission (Macdonald 1956). During the 1965 YF outbreak in Senegal, initial reports from Diourbel quoted the 'density index' in parallel with the Breteau Index (BI) (defined pp.12), where areas below a mosquito density index of 1 (BI = 5) experienced no transmission of the virus (Cornet et al. 1968; Brown 1977). Subsequently in 1967, Soper et al. (1967) reported that a house index threshold of <5% (Soper 1967) was also indicative of low transmission risk. This was later complemented by publication in the Weekly Epidemiological Record by the World Health Organisation (WHO) in 1971 and 1972, where a BI<5, HI<4 and CI<3 were considered low risk for urban YF transmission, while a BI>50, HI>35 and CI>20 were considered high risk (World Health Organisation

1971). These metrics were further reinforced in later texts (Brown 1977) and research articles, where transmission thresholds for dengue were formulated in the same way, and dengue transmission thresholds of 6 (Chen et al. 1994) and 35 (Lin 1994) were recorded. In the 21st Century, it has been noted by Chadee et al. (2009) that YF transmission thresholds have been instrumental in forming those for dengue (Chadee 2009).

The prevailing consensus on dengue transmission is that, whether or not transmission thresholds are derived from YF approaches, these are unlikely to be globally standardised and will be heavily dependent on other context-driven transmission factors, such as circulating herd immunity, human movement, migration and population density, as well as the fluctuating abundance of mosquito vectors (Stoddard et al. 2013; Reiner et al. 2014; Stoddard et al. 2009; Vazquez-Prokopec, Stoddard, et al. 2009; Vazquez-Prokopec et al. 2010).

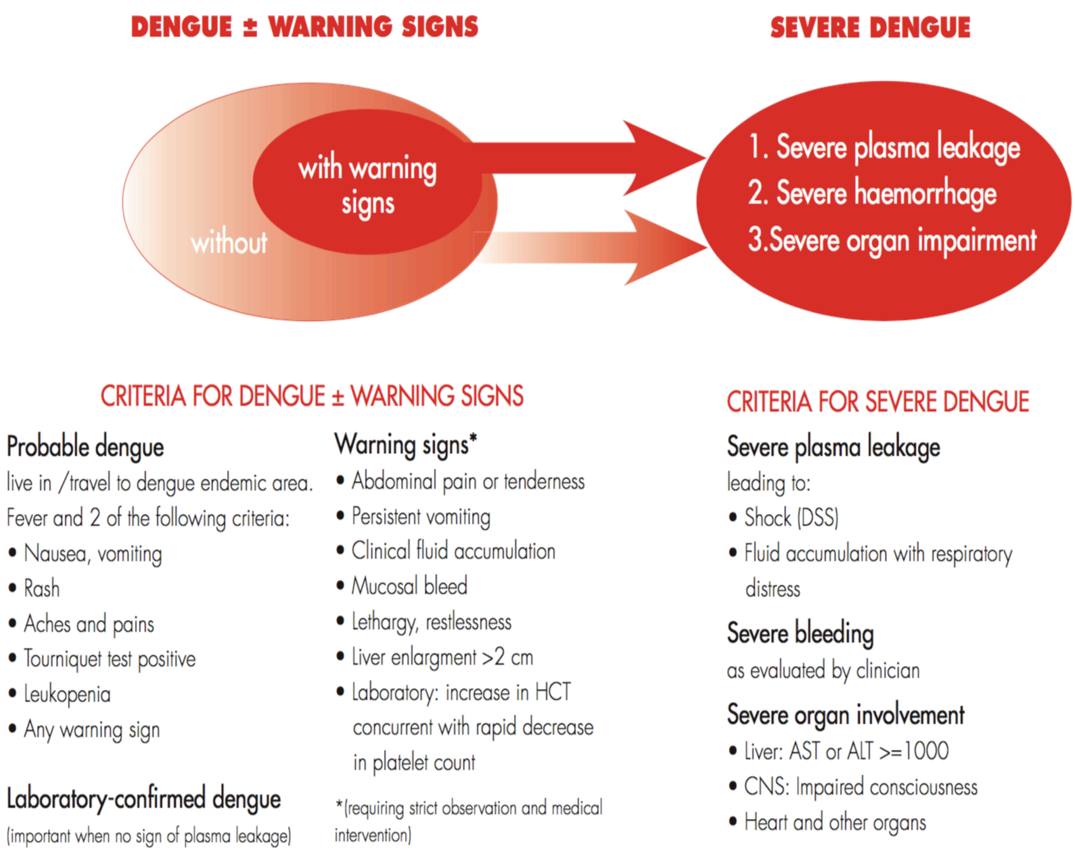
Dengue Clustering

The limited flight range of the *Aedes* mosquito, typically less than 100 metres in dengue endemic settings (Scott & Morrison 2010), contributes to the existence of localised concentrations of the virus (dengue hotspots). Often, positive spatial correlations between the index case and subsequent cases are not found beyond 100 metres (Thomas et al. 2015), further highlighting this tendency for dengue transmission to cluster (Mammen et al. 2008; Van Benthem et al. 2005; Thomas et al. 2015). However in rural settings, inverse spatial correlations persist between the index case and subsequent cases (Van Benthem et al. 2005), perhaps due to lower density housing or increased necessity to move farther from one's immediate neighbourhood on a daily basis. In general, this research adds to a growing body of evidence indicating that the movement of people between areas is more likely responsible for the dissemination of dengue rather than mosquito movement alone (Honorio et al. 2009; Scott & Morrison 2010). Consequently this has led to increased interest in mapping the spatial behaviour of humans as the primary driver for the dissemination of dengue viruses (Vazquez-Prokopec et al. 2010; Stoddard et al. 2009). Coupled with other prevailing transmission factors, such as vector abundance and local herd immunity, dengue transmission still remains a complex issue that requires further work to understand how spatial transmission dynamics underpin endemicity and influence epidemic transmission.

Dengue Diagnosis

Early diagnosis and treatment of dengue can dramatically improve patient recovery, often reducing mortality to almost zero (World Health Organisation 2012a). A combination of clinical symptoms and diagnostic tools are used to classify dengue into dengue or severe dengue, a change from the previous classification of dengue, dengue haemorrhagic fever and dengue shock syndrome (World Health Organisation 1997; World Health Organisation 2012a). This has improved the sensitivity and specificity of clinical diagnoses (Horstick et al. 2015) (Figure 1.3), although some countries still use the 1997 definition. Primary diagnostic tools depend on the detection of genomic/ antigenic viral components, such as the important flavivirus marker NS-1 (non-structural protein 1), virus isolation, or serological markers, including elevated levels of IgG (Immunoglobulin G) and IgM (Guzman et al. 2010). However, some of these tools are less specific than others primarily because: 1) Ig levels can differ between primary and sequential infections; 2) NS-1 antigens are common to all flavivirus infections *e.g.* yellow fever, thus causing cross-reactivity and lower positive predictive values; and 3) some serological tools used to identify dengue infection alone cannot distinguish between virus serotypes (World Health Organisation 2012a; Guzman et al. 2010). Accordingly, case definitions are categorised into probable and laboratory-confirmed dengue (Guzman et al. 2010; World Health Organisation 2012a) and utilise a combination of clinical observations and diagnostic tools. A confirmed dengue case requires either isolation of the virus, detection of antigenic/ genomic components or seroconversion, but the former methods can be expensive and laborious (Ahmed & Broor 2014). Probable case definitions rely on simpler, cheaper methods, such as rapid diagnostic tests to detect the presence of IgM/ IgG, which are often easier to use and more readily available in primary health care settings (Guzman et al. 2010; World Health Organisation 2012a).

Figure 1.3. Dengue case classification (World Health Organisation 2009).



Vector Surveillance and Sampling

Routine vector surveillance is undertaken fundamentally for two reasons: 1) regular surveys over time are used to ascertain discrete relative abundance changes in the circulating vector population (World Health Organisation 2009); and 2) timely relative abundance surveys are used post-intervention for monitoring and evaluation purposes (Focks 2004; World Health Organisation 2009). The requisite surveillance differs in both contexts. Depending on the sampling approach, large sample sizes are needed to detect small, often seasonal differences in the circulating population (Focks 2004). Arguably, the best approach for this is to sample eggs, and perhaps larvae, as they are far more abundant than other life stages (Focks 2004). In contrast, for monitoring and evaluation, where the expected difference between control/ baseline and intervention is high, sampling pupae and adult mosquitoes is the optimal way to determine impact, given that the relationship between earlier life stages and the adult mosquito remains unknown (Focks 2004) and fewer numbers would be required. Additional outcomes of interest, such as mosquito virus infection rates (Borsboom & Boatin 2003) or the age of the circulating adult mosquito (Styer et al. 2007) population are also useful for surveillance purposes.

Contemporary Limitations

The overarching problem with dengue vector surveillance, either for routine quantification or for the monitoring and evaluation of control programmes, lies in the multiple forms of sampling error inherent within the process (Scott & Morrison 2010; Horstick et al. 2010). These include the following:

- Non-standardised sampling strategies (Williams et al. 2006; Horstick et al. 2010).
- Human error in sampling activities/ data input and analysis (Wilson et al. 2015).
- Presence of the vector in both domestic and peri-domestic areas.
- Access to mosquito populations, especially endophillic mosquitoes (Williams et al. 2006).
- Inadequacy for indices to sensibly capture prevailing abundance measures (Focks 2004; Williams et al. 2006).
- Absent algorithms used to relate different life stages.

The diurnal, endophillic nature of *Aedes* vectors presents another problem for surveillance: that transmission can take place both in the home and the workplace, including other public places such as hospitals, schools and parks (Scott & Morrison 2010). The ubiquitous distribution of the vector across these settings requires large scale surveillance activities, but due to funding constraints, this is almost always out of reach (Horstick et al. 2010). Often, smaller neighbourhood areas become the focus of surveillance efforts, but this practice results in irregularities in data capture, geographic coverage and temporal monitoring, all of which contribute to poorer datasets and less robust surveillance systems. Not to mention that arbitrary surveillance is time consuming and costly (Horstick et al. 2010). In addition to these limitations, mosquito abundance is dependent on land use *i.e.* urban areas provide an increased number of breeding sites for *Ae. aegypti* than rural areas, which can be highly variable across space. Clearly, where financial resources are abundant, it is preferred that sampling strategies systematically target all buildings within a given area (including places of residence, recreation and work) to adequately capture a representative sample of the resident mosquito population (Focks 2004).

Ovitrap Metrics

Ovitraps are a range of devices that are used to either collect information on presence of *Aedes* species by luring mosquitoes to deposit eggs, or simply to attract adult mosquitoes for extermination, known as lethal ovitraps (Harwood et al. 2015; Ritchie et al. 2008; Rapley et al. 2009; Zeichner & Perich 1999). To be effective, ovitraps must contain water in a bid to replicate natural breeding sites (Zeichner & Perich 1999). They may also contain olfactory and visual attractants, such as CO_2 (Harwood et al. 2015) or hay infusion (Perich et al. 2003), and/ or darkly coloured materials (Ritchie et al. 2008; Zeichner & Perich 1999). They are widely used in regular surveillance in many locations worldwide and are recommended by WHO for this purpose (World Health Organisation 2009). Although it is common to count the eggs in ovitraps as a measure for vector abundance, it has been demonstrated that such uses may lead to calculations that are highly inaccurate (Focks 2004), in part due to the preference for *Aedes* vectors to perform skip oviposition, loosely defined as a preference for the gravid female to lay eggs in multiple batches across a number of breeding sites, rather than all eggs in a single batch in one location (Williams et al. 2008). Consider also that ovitrap indices rely firstly on the total number of ovitraps as the denominator in many calculations, and secondly, albeit indirectly, rely on the absolute number of prevailing alternative (or cryptic) containers (World Health Organisation 2009), which may vary from neighbourhood to neighbourhood *i.e.* few available alternative breeding sites may increase the number of eggs observed in ovitraps, or an increased number of positive ovitraps may simply represent a tendency for female mosquitoes to increase skip oviposition rates due to greater breeding site availability (Focks 2004). In either scenario, it is clear that the number of eggs cannot be taken as a true cross-sectional measure of prevailing adult mosquito abundance. Yet, there remains sufficient evidence for the application of ovitraps to monitor low level vector abundance, the impact of insecticide treatments and/ or the seasonality of adult abundance within homogeneous areas (Focks 2004; Focks et al. 1987).

Larval and Pupal Indices

The sampling of larval/ pupal stage dengue vectors is a fundamental part of all dengue surveillance programmes worldwide. Originating in yellow fever vector surveillance, mosquito metrics were established to quantify the breeding density of the vector as a proxy for adult abundance and therefore transmission risk (World Health Organisation 1971). This approach has proved popular for *Aedes* vectors due the relative ease of data capture when compared with adult or ovitrap sampling techniques. Indeed, sampling protocols are well established, easily followed and available from WHO (World Health Organisation 2009).

There are different methods available to sample the immature stages. These include floating traps (Harrison et al. 1982), often termed funnel traps, which allow practitioners to estimate the number of pupae or larvae in particular breeding sites, but only after parameterisation using identical breeding sites and known numbers of immatures (World Health Organisation 2009). Or, one can directly observe the presence of immatures in household containers to calculate the standard *Stegomyia* indices (Focks 2004). Such indices often ascertain the ratio of positive containers or number of pupae to the number of houses or number of people respectively (World Health Organisation 2009). These can be used to quantify the relative abundance of *Aedes* immatures at any one point in time but are a poor correlate to adult abundance (World Health Organisation 2009).

The *Stegomyia* indices are among the most commonly used for vector surveillance, and where the household is the sampling unit, the indices can be described as the following: Container Index = percentage of water-holding containers infested with larvae or pupae; House Index = percentage of houses infested with larvae and/or pupae; Breteau Index = number of positive containers per 100 houses inspected (World Health Organisation 2009). The calculations of each are presented below:

HI = % of infested houses positive for larvae/pupae (World Health Organisation 2009)
CI = % of water-holding containers positive for larvae/pupae (World Health Organisation 2009)
BI = No. positive containers per 100 houses inspected (World Health Organisation 2009)

While these basic formulae are simple and easily used in operational capacities, there are disadvantages. Arguably the most important of these is that each of the standard *Stegomyia* indices fails to account for variation in container productivity, *i.e.* the number of larvae/ pupae that emerge as adult mosquitoes per container, as surveys only report containers or houses positive for immatures. This is an important omission which can lead to inaccurate measures of dengue risk (Focks 2004; Manrique-Saide et al. 2008), as larval survival is density dependent (Focks 2004). Indeed, larval survival is also a function of the quality and size of the breeding site – larger water bodies are often more stable in terms of temperature and nutrient supply and are indeed preferred vector breeding sites (Focks et al. 2006; Harrington et al. 2008). As this variation affects the chance that immature stage *Aedes* will complete the cycle to adulthood (Manrique-Saide et al. 2008), assessing the productive capacity of container types is an important consideration - detail which is lost among the *Stegomyia* indices. To address this, 'container productivity assessments' have been used to identify and subsequently target for control those containers that are responsible for a large number of mosquito adults. To do this, L4 stage larvae and/ or pupae are quantified, because these life stages represent the closest immature form to an adult mosquito (Focks et al. 2006; Manrique-Saide et al. 2011; Manrique-Saide et al. 2008). Then, the type of container in which the immatures were found e.g. flowerpot, drum, is also recorded (Manrique-Saide et al. 2011). Once the survey is complete, the proportion of pupae stratified by container type can be ascertained (Focks & Chadee 1997). Evidence has shown that this method is useful to identify large productive containers (Midega et al. 2006), as well as focus vector control strategies (Maciel-de-Freitas et al. 2007), however more recent research has demonstrated that still targeting all container types has a higher impact on mosquito densities over the long term (Maciel-De-Freitas et al. 2009; Maciel-de-Freitas & Lourenço-de-Oliveira 2011).

Adult Mosquito Indices

In parallel with the *Stegomyia* indices, adult metrics for *Ae. aegypti* were also conceived during the yellow fever era, when metrics such as the vector biting rate (bites per man hour (>2 bites/ man hour was considered high risk)) were commonplace (World Health Organisation 1971). The primary means for generating these metrics was the employment of people to expose themselves to biting mosquitoes, called the human landing catch (HLC). Clearly, this involved a degree of risk for the human concerned, given that exposure to disease was also possible. While this practice was widespread, these metrics were likely less accurate than once thought, as biting rates are dependent upon individual human characteristics, such as the microbial flora of the skin (Verhulst et al. 2011) as well as the individual's ability to catch mosquitoes. Updated ethical guidelines now ensure that this practice cannot be undertaken where the risk of exposure to disease is present (Williams et al. 2006). Consequently, the remaining possible sampling strategies for adult mosquito sampling and surveillance are either the direct aspiration of resting mosquitoes, or passive, attractant/ non-attractant mosquito traps (SantAna et al. 2014).

Non-attractant traps have the advantage of sampling the general mosquito population without bias towards a particular species that may respond more favourably to the presence of attractants; that said, non-attractant traps tend to sample the immediate vicinity surrounding the trap, rather than drawing insects from outside this immediate zone (Service 2008). At least in this regard, attractant traps are more favourable. Such traps use CO_2 as the attractant, however this can be expensive to obtain and difficult to dispense in the field (Williams et al. 2006). And while light traps have also demonstrated success with various mosquito species (Service 2008), they are generally not as effective at collecting high proportions of *Ae. aegypti,* especially when compared with manually aspirated collections using the CDC-backpack aspirator; indeed, this is perhaps not surprising as this species is a day biter. Furthermore, intense light at a short range can actually repel mosquitoes (Service 2008), thus further reducing the sampling capacity of these traps. While human-landing catches are no longer employed, and despite contemporary evidence on human variation in attractivity, they were regarded as highly effective (Schoeler et al. 2004). As a result, novel methods to capture the adult were commonly evaluated against human landing catches, which led to the vindication of mosquito aspiration methods as a viable sampling tool, as they were found comparable to the sensitivity of human landing catches, and better than the majority of attractant and non-attractant traps (Schoeler et al. 2004; Clark et al. 1994; Williams et al. 2006).

Given that blood-fed *Ae. aegypti* tend to rest in homes, manually aspirating them is the natural choice. Indeed, this method collects a higher proportion of blood-fed females when compared with attractant traps, which is an important consideration if investigators seek to ascertain the infection status of the mosquito population (Williams et al. 2006). But while adult sampling using aspirators has proved effective, limitations with this method exist. One of the first standardised aspirators, the CDC backpack aspirator, has been widely used since inception (Clark et al. 1994), yet it is heavy, restrictive and expensive (Maia et al. 2011). Equally, non-standardised operators tend to bias the results – a problem that does not affect standalone independent sampling traps. Indeed, such cumbersome, bulky and noisy equipment can lower community acceptance; unsurprisingly, these combined factors have, until now, hindered the widespread adoption of this sampling method (Williams et al. 2006; Maia et al. 2011). Fortunately, newer technologies have been developed, particularly in the form of the Prokopack aspirator (Vazquez-Prokopec et al. 2009). This aspirator is lightweight, easy to use, cheap to build and repair, and aspirates mosquitoes efficiently (Maia et al. 2011). When considering absolute mosquito numbers, the Prokopack aspirator is also able to reproduce similar catch numbers as the CDC backpack aspirator, as well as increase the consistency of collections between technicians and achieve higher user-friendliness ratings (Maia et al. 2011).

In light of the recent evidence demonstrating that adult mosquito sampling has better temporal associations with dengue incidence than either larval or ovitrap sampling (de Melo et al. 2012), coupled with the clear advantages over passive sampling traps, and recent advances in technology, aspirating mosquitoes is now the recommended sampling method for *Ae. aegypti* (Scott & Morrison 2010; Achee et al. 2015).

Virus infection rates

Without the capacity to test for mosquito infections, a method to evaluate the true impact of vector control on interrupting transmission between vector and human (and not only the absolute or relative vector densities), would be lost. Monitoring vector virus infection rates can have a number of advantages over standard adult/ larval abundance data. Firstly, geographic identification of localised mosquito infections can provide a measure of risk to the human population using metrics such as the annual transmission potential, often observed in other vector borne disease systems (Hati et al. 1989; Borsboom & Boatin 2003). Indeed, this method can be used to evaluate the impact of control programmes using a before-and-after epidemiological study design (Borsboom & Boatin 2003). Secondly, metrics reflecting current vector infection rates allow the localised targeting of vector control tools, prior to possible outbreaks (Eisen et al. 2009). This is currently used with success in other arboviral models, such as West Nile Virus (WNV) transmission, and eliminates the need to test for virus infection in other animal reservoirs (Burkhalter et al. 2006; Voge et al. 2013). However, virus infection rates are often low, and necessitate large sample sizes (Eisen et al. 2009).

While infection rates in mosquito vectors such as *Anopheles* mosquitoes are easily quantified by microscopy of blood-meals from infected individuals (Ndiath et al. 2014), detecting the dengue virus in either individual or pooled (<100) *Aedes* is a time consuming and expensive process (Voge et al. 2013; Eisen et al. 2009). An alternative is to isolate or extract the virus RNA via RT-PCR, but this is often not available to surveillance laboratories in low-income countries, mainly due to the large capital required to purchase and maintain reagents and equipment, as well as train personnel (Voge et al. 2013; Muller et al. 2012). Yet, breakthroughs in this field are emerging: NS-1 rapid diagnostic tests now demonstrate an ability to determine infections in both individual and pooled mosquitoes (Voge et al. 2013; Tan et al. 2011; Muller et al. 2012); field-based PCR (Polymerase Chain Reaction) platforms are a reality and will likely become more widely available in the future (Pal et al. 2015). Considering this progress, the future of vector surveillance may ensure that control efforts and resources are better targeted to those areas where dengue virus is currently circulating amongst the mosquito population, leading to more timely responses and efficient surveillance methods to monitor and evaluate vector control programmes, which will complement epidemiological outcomes.

Mosquito Age Distribution

The age distribution of circulating adult mosquitoes is a consideration for the force of dengue infection, due to a multiplicity of factors. An intuitive consideration for disease vectors is that, with age, the vector is more likely to acquire an infection (Styer et al. 2007), as this is a function of the probability of infection during blood-feeding multiplied by the number of exposures. Additionally, increased mosquito population age increases the likelihood that infected mosquitoes will deliver more infective bites. Therefore it follows that younger vector populations have a lower vectorial capacity than older populations. However, this view fails to take into account age-dependent fitness costs. These can manifest in different ways, from limiting mosquito flight potential (Nayar & Sauerman 1973), altering immune function (Christensen et al. 1986) to ultimately, increased mortality, a process known as senescence (Styer et al. 2007). Couple these considerations with the knowledge that the fitness of vector populations infected with DENV or indeed other infectious agents is also adversely affected - as observed in the feeding time and fecundity of female *Ae. aegypti* (Sylvestre et al. 2013) and parasite systems elsewhere (Styer et al. 2007) - the force of infection and vectorial capacity become more complicated to quantify. While these are important theoretical consideration, many have argued that these processes would not normally impact wild-type mosquitoes, mainly as survival has not been predicted to last beyond the second gonotrophic cycle (MacDonald 1952; Styer et al. 2007). Given these complex considerations, it is clear that mosquito adult age is only one of a number of covariates that could be used to define transmission risk, and that further research in this area is needed to identify the importance of mosquito population age profiles as a factor in vectorial capacity, the force of infection and ultimately in dengue transmission.

Contemporary Outlook

Mosquito surveillance is undertaken across much of the world (Horstick et al. 2010; Pilger et al. 2011). As discussed, there are many factors that contribute to the effectiveness of surveillance programmes. There still exist some important questions that must be answered for the scientific community to adequately predict dengue outbreaks. Firstly, whether surveillance technologies and practices can accurately represent the true characteristics of the circulating mosquito population. And secondly, whether these surveillance programmes are currently used to warn of forthcoming dengue outbreaks and indeed whether such methods are effective in doing this. Coupled with the huge financial and human resources required for these practices, a systematic review is desperately needed to identify the strengths and weaknesses of existing surveillance campaigns, and indeed whether mosquito indices can be used as predictors of forthcoming dengue transmission.

Current Dengue Vector Control

Existing Vector Control Strategies

Currently, interventions against dengue transmission target the mosquito vector, as this is the only preventative form of dengue control available. Evidence for the success of these interventions is generally either limited in number and/ or by epidemiological study design (Esu et al. 2010; Erlanger et al. 2008; Horstick et al. 2010; Pilger et al. 2010). Such a poor evidence base for vector control interventions is most likely because epidemiological trials that evaluate vector control interventions suffer from constrained budgets and inadequate intervention coverage, leading to unsuccessful attempts at controlling the vector (Lambrechts et al. 2015; Morrison et al. 2008). Considering this, further evidence is required to support or discourage the use of vector control tools to limit both seasonal and epidemic dengue transmission.

There are a number of vector control methods available to the practitioners, which include horizontal and vertical approaches that may be insecticidal, genetic or environmental, split between those that target the mosquito adult and/ or immature life stages. Targeting the mosquito immature stages is central to many dengue vector control programmes, and given the high number of possible breeding sites throughout communities, these interventions are best implemented at the community level. Unsurprisingly, success of these interventions is highly reliant on the coordination and commitment of those involved (Horstick et al. 2010; Lloyd et al. 1994; Azmawati et al. 2013). There are various types of community-based interventions, but most use a combined approach utilising ITMs (insecticide-treated materials), residual insecticides in the form of repellents, clean up campaigns, water covers, and the formation of social structures, such as Community Working Groups (CWG) World Health Organisation 2012a). At the same time, vertical approaches, usually led by NGOs (non-governmental organisations) and/ or the local government, frequently aim to educate the local population and raise awareness of dengue symptoms, as well as the vector life cycle (Horstick et al. 2010). Yet, all dengue interventions, either vertical or community-based, and insecticidal or mechanical, have met with mixed success. For example some studies show an impact on entomological indices using a range of community-based methods (Vanlerberghe et al. 2010) however there are contrasting results from more recent eco-bio-social studies (Sommerfeld & Kroeger 2012). Indeed, the most recent review on the effectiveness of vector control suggests that a myriad of activities have no demonstrable effect on entomological indices (Horstick et al. 2010). In fact, four studies even reported an increase in dengue cases despite intensive vector control campaigns (Horstick et al. 2010). Yet, in light of the absence of an available vaccine and while novel technologies are under evaluation, public health authorities and vector control campaigns must continue to use any means available to combat the vector and subsequent transmission (Guzman et al. 2010).

Insecticide fogging or space-spraying

Fogging/ space spraying requires the use of heavy equipment to deliver an aerosolised dose of insecticide to any nearby flying insects, including the vectors of dengue (World Health Organisation 2012a). The intervention can be delivered both indoors and outdoors by teams using specialised equipment (World Health Organisation 2012a). It is a highly visible approach and regularly used by health authorities during dengue transmission, especially epidemics, as a means to rapidly eliminate circulating adult mosquitoes that may be infected with dengue – this is despite a lack of 'level 1/ gold standard' evidence for effectiveness (Beatty et al. 2010; Harrington et al. 2013; Esu et al. 2010). For this reason, the term 'political fogging' has been used in some circles to describe a reliance on this method to demonstrate action and involvement of the local government in the outbreak (Horstick et al. 2010).

Fogging can be conducted either on foot or by vehicle. Outdoor fogging is easy to apply and unconstrained by household acceptability, thus coverage of targeted areas is often easier and more complete. However, this approach may not reach inside homes and public buildings, which is where blood-fed vectors, and hence likely infectious mosquitoes, tend to rest (Renganathan et al. 2003; Perich et al. 2000; Schoof 1967). Because of this, there is much debate around the effectiveness of this approach. In spite of its popularity, little evidence exists to support the effectiveness of outdoor fogging against both the mosquito population and dengue transmission. It has been assessed in Erlanger *et al*. (2008) as effective at reducing the Breteau Index (BI) (Erlanger et al. 2008), and when used peri-domestically, was able to reduce immature indices for short periods in 13/15 studies (Esu et al. 2010). In addition, recent evidence demonstrated that various formulations result in 100% mosquito mortality at 10m distance, and >50% mosquito mortality at a distance of 50m, despite dense vegetation (Karunaratne et al. 2013). However, mosquito mortality was assessed using caged mosquitoes, which are not able to move away from irritant/ lethal chemicals. Finally, evidence from Thailand suggests that this approach is inadequate for reducing the number of 'secondary' dengue cases, defined as cases arising within 100m of the index case within 16-35 days of onset (Thammapalo et al. 2012), but this observation is overshadowed by poor implementation of WHO standardised protocols and indeed the movement of people, rendering precision in defining the origin of dengue infection difficult. While considered less practical than outdoor fogging for reasons of social unacceptability and residential access (World Health Organisation 2012a) indoor fogging is still preferred for targeting infectious blood-fed mosquitoes. Indeed, this method has proved more effective than outdoor fogging in quasi-experimental field conditions, where mortality was 100% and 80% respectively (Loke et al. 2015). Even so, in this trial mosquitoes were caged and therefore could not move away from the insecticide formulations, thus it is difficult to infer the true operational implications of these interventions. To this date, very little evidence is available in support of indoor fogging as a singly effective intervention (Erlanger et al. 2008). In summary, such a limited evidence base precludes the recommendation of fogging in any capacity as a standalone intervention (Esu et al. 2010; Pilger et al. 2010).

Residual Spraying

Perifocal spraying is used to cover the area surrounding the home, including in and around containers, with residual insecticides such as temephos. It was predominantly used in combination with source reduction to great effect during the Yellow Fever (YF) eradication campaign in the 1960s (Achee et al. 2015). It has also seen success in the Cayman islands (Nathan & Giglioli 1982), again as part of a combination approach with indoor and outdoor residual spraying, and in Australia, when used tyres were targeted to effectively diminish the larval population (Nguyen et al. 2009). However, such examples cannot be used to support perifocal residual spraying as a standalone control tool, as most interventional research includes the use of additional control tools in a combined approach. Thus any inferences of the true effect of this intervention must be treated with caution.

By contrast, indoor residual spraying is used to combat predominantly endophillic vectors of human disease. For mosquito control, the intrinsic value of IRS (Indoor Residual Spraying) is in its ability to apply an active, residual insecticide, sustained for long periods, which reduces not only the abundance of adult mosquitoes, but also the age of the circulating population, thereby further limiting the chance for transmission (Scott & Morrison 2010). The insecticide is applied to walls and ceilings inside buildings, most often houses, remaining effective for up to 5 months and longer, depending on the surface type (N'Guessan et al. 2010; Tangena et al. 2013). However, although successful against malaria vectors, the impact of IRS on dengue mosquitoes remains uncertain, as the vectors tend not to rest on walls, preferring instead furniture, fixtures and fittings (World Health Organisation 2012a). Despite this, IRS is regaining status as a preferred method of insecticide delivery for dengue control (Chadee 2013), with notably improved results (Vazquez-Prokopec et al. 2010). Recently, it is has been used in outbreak response programmes, notably in Queensland, Australia (Ritchie et al. 2013), and is gaining vocal support of the dengue community (Achee et al. 2015). Yet, few randomised controlled trials have assessed its effectiveness against *Ae. aegypti* (Ellis et al. 2011; Esu et al. 2010; Badurdeen et al. 2013).

Insecticide Treated Materials

Insecticide treated materials (ITM) including curtains (ITC) and water covers (ITWC) have met with some success. In Kroeger *et al*., 2006, pronounced effects against the vector were observed when compared to baseline, however, due to a proposed community effect, reductions were also observed in control clusters, rendering differences between the two arms statistically insignificant (Kroeger et al. 2006). And in Lenhart *et al*., 2013, consistent statistically significant differences were notably absent between intervention and control clusters (Lenhart et al. 2013). Elsewhere, ITCs have fared better. Where ITC coverage was highest, evidence of a significant reduction of intradomicillary dengue transmission and reduced dengue-infected *Ae. aegypti* populations was observed (Loroño-Pino et al. 2013). Similar effects were demonstrated in Vanlerberghe *et al*., 2013, wherein authors reported a significant reduction in the Breteau Index after 6 months, but only when coverage was 70.5% (Vanlerberghe et al. 2013). Impact reduced to statistically insignificant levels when coverage decreased to 33.2% after 18 months (Vanlerberghe et al. 2013). Thus, the debate on the use of the myriad of ITMs as a viable, evidence-based method to control dengue still requires further research.

Biological Control

Biological control is considered particularly popular due to the absence of reliance on broad-spectrum insecticides, and in this sense is a particularly 'green' approach. In addition, while vertical implementation is feasible (indeed, necessary for the generation of large quantities of biological control agents), usually the community administers this approach, leading to localised vector control and empowerment of discrete human populations. However, the evidence in support of biological control methods is often mixed, while limitations regarding sustainability and acceptance abound (Lazaro et al. 2015; Han et al. 2015). Larvivorous copepods are often cited in Vietnam as a particularly effective control method, in fact so effective that it also reduces dengue incidence (Vu et al. 2005; Kay & Nam 2005; Nam et al. 2012). In Vu *et al*., 2012, authors describe the success of *Mesocyclops* application to water storage containers in reducing larval 3rd and 4th instars (Vu et al. 2005), yet questions remain over the applicability of such treatments across other contexts, and success with this approach has not been proven elsewhere (Lazaro et al. 2015). Indeed, in these studies, other interventions, such as source reduction, also ran simultaneous to the application of copepods, making it impossible to analyse the true effect of copepod distribution. Furthermore, operational restrictions, such as the necessary re-application of copepods to containers, drastically reduce the potential for this method as a long-term sustainable control tool (Lazaro et al. 2015). Authors of a recent review suggest that the lack of effectiveness of this approach in other contexts is likely due to poor community-participation (Lazaro et al. 2015), probably because drinking water must be seeded with these agents, which understandably breeds suspicion and poor acceptance among communities. Ultimately, the review concludes that there is no strong evidence to recommend copepods as an effective biological control method against dengue vectors, given the widespread failure of this approach in many other country contexts (Lazaro et al. 2015). A notable omission from both the review and indeed research promoting this as an effective vector control tool is that *Mesocyclops* are themselves vectors of other human disease, notably gnathostomiasis (Janwan et al. 2011) and dracunculiasis (Cairncross et al. 2002), which cause considerable long-term problems amongst a minority of human populations.

Historically, fish have been used synergistically as a form of biological control in rice paddies. Indeed, fish are known to predate on mosquito larvae (World Health Organisation 2009), while those that predate on *Aedes* larvae include guppies, perch and carp (Han et al. 2015). Accordingly, this approach is listed as a potential dengue control method by the World Health Organisation (World Health Organisation 2009). However, in a recent review, the evidence-base in support of these methods was critically assessed and demonstrated that there is: 1) very weak, limited evidence in support of this approach to reduce dengue incidence; 2) no evidence to support this as an effective community-level tool to reduce *Aedes* larvae; and 3) only experimental evidence to support this as a viable approach in some container types (Han et al. 2015). Indeed, until further evidence emerges, this method should not be recommended as a reliable method of dengue vector control at the community level, in line with recent research confirming that this method is also not useful for the control of *Anopheles* larvae to reduce malaria incidence (Walshe et al. 2013).

Topical Repellents

Human populations endemic for vector borne diseases, as well as travellers to endemic regions, have historically used topical repellents to ward off blood-sucking insects. There are a myriad of laboratory and semi-field studies demonstrating a repellent effect against different heamatophagus mosquitoes, including *Ae. albopictus*, using various insecticidal compounds (Chattopadhyay et al. 2013; Kalyanasundaram & Mathew 2006; Yap et al. 1998; Ogoma et al. 2012). Indeed, recent evidence for a variety of effective natural and synthetic compounds against *Ae. aegypti* is beginning to emerge (Sanghong et al. 2015; Yu et al. 2015; Kumar 2014). As with most vector control, the effect of these compounds must be two-fold: 1) they must reduce the mosquito-human biting/ contact rate; and 2) they must limit transmission and thereby reduce the incidence of disease. A recent systematic review and meta-analysis of the effect of topical repellents on malaria incidence evidenced no effect in reducing malaria cases (Wilson et al. 2014). Indeed, no epidemiological studies have been conducted on the effect of topical repellents on dengue incidence, thus this field is awaiting new research.

Integrated Vector Management

The combination of vector control tools to optimise allocation of resources is often cited as the most practical and cost-effective vector control method (World Health Organisation 2012a). Indeed, integrated vector management (IVM) emerged as most effective at controlling dengue vectors in the review article by Erlanger *et al*., 2008 (Erlanger et al. 2008), and is widely recommended by the international community (World Health Organisation 2012a; World Health Organisation 2015). In a recent review, integrated control methods were found to be more effective than standalone control methods (Lima et al. 2015). Considering this, the following guidelines are considered important when conducting IVM:

- Empowerment of communities, advocacy and social mobilisation (World Health Organisation 2015).
- Inter- and intra-sectoral collaboration (World Health Organisation 2015).
- Integration of mixed vector control methods alongside allied disease control measures (World Health Organisation 2015).
- Data driven decision making through operational and academic research into epidemiological and entomological surveillance and evaluation (World Health Organisation 2015).
- Capacity building by developing the structure for adequate training and hire of necessary personnel at both national and local levels to manage and promote integrated vector management programmes (World Health Organisation 2015)

Indeed it is highly likely that integrated management techniques will be adapted to include vectors of additional diseases, especially where synergies between certain vector-borne diseases are clearly apparent. Recent research suggests that these synergies would be utilised for optimal use of resources in both research and operational management (Golding et al. 2015).

Insecticide resistance

In an uncomfortable parallel with the spread of antibiotic resistance, global insecticide resistance is becoming more prevalent across a number mosquito species and insecticide classes – two classes in particular: pyrethroids and organophosphates (Ranson et al. 2010; Rodriguez et al. 2007). Resistance to these insecticides is largely seen in mosquitoes and black flies, but less so in other vectors, where reproductive rates are slower (tsetse flies and triatomine bugs) (Hemingway & Ranson 2000; Andrade & Junior 1990). This is predominantly due to the nature of mosquitoes as *r* species, – those that reproduce quickly and in large numbers – as beneficial evolutionary genotypes are able to emerge within populations due to the sheer weight of numbers over short time periods. Indeed, mosquito species have been exposed to insecticidal pressures for a number of years from varied sources and arguably these selection pressures are the result of a combination of factors: 1) widespread use of these insecticides in the agricultural sector (Overgaard 2006) 2) sole reliance on these classes to form the foundation of a wide variety of vector control interventions (Ranson et al. 2010), and 3) an abundance of poor quality spray formulations (World Health Organisation 2001). Clearly, the emergence of insecticide resistance in medically important vectors, in particular mosquitoes, has major consequences for the incidence of vector borne disease (Ranson et al. 2010). As a result, novel formulations and alternate strategies to disseminate insecticides are needed and are currently under development (Ranson et al. 2010; Hemingway et al. 2006; Marcombe et al 2011).

Ae. aegypti is demonstrably resistant to DDT, following large scale control programmes in the Caribbean and Latin America, and is becoming increasingly resistant to pyrethroids and organophosphates, notable for their use in space spraying, insecticide treated nets and materials, larvicidal applications and residual spray formulations (Ranson et al. 2010; Rodriguez et al. 2007; Marcombe et al. 2011; Sivan et al. 2015; Ayorinde et al. 2015; Koou et al. 2014). Worryingly, emerging resistance to carbamates and organochlorines has also been documented (Ranson et al. 2010), although this is not yet as widespread as resistance to the former two classes. Temephos, an organophosphate with low mammalian toxicity, was widely used against the larvae of *Ae. aegypti* during early control programmes and remains in use today, however resistance in this vector has become common throughout many Latin American strains of *Ae. aegypti* (Alvarez et al. 2014; Rodriguez et al. 2007). Recent research elsewhere has also documented resistance to other organophosphates, and indeed carbamates (Koou et al. 2014), possibly due to widespread use of these in domestic preparations and against other insects in urban areas. Clearly, in terms of controlling the vector, this has a knock-on effect. Indeed, in some situations, resistance mechanisms have reduced the effectiveness of vector control tools (Grisales et al. 2013; Marcombe et al. 2011). As a result, it has even been argued that some control measures that result in sustained exposure to insecticides, should be reduced (Luz et al. 2011). Luz *et al.* (2011) argue that approaches such as continuous larval control actually contribute to emerging resistance, thus negatively impacting the effectiveness of adult mosquito control, thereby reducing the cost effectiveness of these outbreak control tools (Luz et al. 2011). Yet, it still remains unclear whether emerging and prevalent insecticide resistance will negatively impact entomological outcomes in the field, and thereby have a knock-on epidemiological impact, as observed for *Anopheles* (Strode et al. 2014).

As with all insecticide-based approaches, there should be available means to detect increasing resistance and reducing effectiveness over time (Hemingway et al. 2006; Ranson et al. 2010; Russell et al. 2014). To combat emerging resistance, insecticide resistance should first be quantified. Standardised insecticide resistance bioassay methodologies are well documented among the international community (World Health Organisation 2013), while the recently developed insecticide quantification kit can be used to take a snapshot of the prevailing insecticide dosage on a wall at any particular time point (Russell et al. 2014). Additionally, mixtures of insecticides have been trialled with some success and will likely gain traction as a method of preserving the effectiveness of single insecticides (Marcombe et al. 2011). However, only future research will discover whether the combination of these approaches were effective in reducing selection pressures for standalone insecticides among mosquito vectors.

Future Dengue Prevention and Vector Control

Spatial Repellents

Basic spatial repellents, such as pyrethrum 'mosquito coils' or traditional materials are available worldwide and have been used for generations. Today, many are still widely used by householders, indicating that populations remain amenable to such strategies (Paz-Soldan et al. 2011).

Based on this desire for repellents, there has been renewed interest in 'spatial repellents' and recent research has pursued the development of novel repellent formulations to repel mosquitoes from the entire home. This forms one side of what is called the 'Push-Pull' strategy, where mosquitoes are repelled or 'pushed' from the home and lured or 'pulled' to attractant lethal mosquito traps, thereby altering the behaviour of the mosquito leading to a reduction in circulating adult mosquitoes (Wagman et al. 2015; Paz-Soldan et al. 2011).

In experimental settings, 58% and 70% of mosquitoes were deterred from entering experimental huts using metofluthrin coils and DDT-treated fabric respectively (Achee et al. 2012). Importantly, this method utilises insecticide quantities far lower than necessary for toxicity (Achee et al. 2012), suggesting that repellency is being achieved. In support of this, lab studies have also shown dosage-dependent repellent effects (Chattopadhyay et al. 2013). However, it remains to be seen whether repellent interventions are able to impact mosquito behaviour prior to blood feeding, and indeed whether this would affect dengue transmission (Manda et al. 2013). Significantly, feasibility studies would also need to be conducted and as yet remain absent (Chattopadhyay et al. 2013).

The ‘pull’ part of the ‘push-pull’ mechanism still requires further research. While evidence behind attractant technologies and reagents for dengue vectors is strengthening (Harwood et al. 2015; Hoel et al. 2015), these are largely laboratory-based or ‘proof of concept’ studies. Field-based evidence is urgently needed to assess the operational impact of such mechanisms on dengue incidence among human populations (Achee et al. 2012).

Genetic Modification and Wolbachia

Vector control has entered a new era, at least with regard to genetic manipulation and infection of mosquitoes. As genetic modification techniques have evolved, so too has their application. A notable example currently deployed in research capacities worldwide is based on a variation of the sterile insect technique (Alphey et al 2012), defined as a method to limit the reproduction of wild-type mosquitoes through the release of genetically engineered or biologically altered (irradiated or infected) mosquitoes (Oléron-Evans–2014). Researchers genetically engineer and release male mosquitoes with a dominant lethal gene (RIDL (Release of Insects with Dominant Lethality) that kills resultant offspring (Alphey & Alphey 2014). This is a highly specific means of targeting the dengue vector (Miller 2011), especially considering that most insecticide-based technologies indiscriminately impact insect ecology, while promoting unwanted resistance (Alphey & Alphey 2014). Specifically, the technique prevents the development of the f1 generation from larval to pupal form, leading to the death of the larva (Alphey & Alphey 2014) and in this manner, is able to dramatically crash mosquito populations. In the context of an outbreak, this would mean destroying the next generation of mosquitoes thereby disrupting the cycle of human-vector transmission (Harris et al. 2012). However, the approach does not target those adult mosquitoes already infected and currently transmitting dengue, while it can be costly due to the intensive labour requirements of separating newly emerged males from females, prior to release. Field trials are on-going.

A second novel approach utilises symbiotic bacteria that are known to render mosquitoes refractory to dengue infection, and thus act as a 'dengue vaccine' for mosquitoes. *Wolbachia pipientis,* an endosymbiotic bacterium, is able to reduce adult *Ae. aegypti* longevity and interrupt dengue infection (Walker et al. 2014). In addition, the bacterium can be transmitted vertically to next generations (Lambrechts et al. 2015). This technique could rapidly disrupt dengue infection in wild type mosquitoes, thus serving as a control tool for mosquito populations, but crucially, with the possibility of long-term fixation in the vector population (Hoffmann et al. 2011). However, the technique has not yet been deployed operationally and still requires further evaluation, preferably in a randomised controlled setting (Lambrechts et al. 2015). However, due to the cost of this approach, a number of observational studies are currently proposed as a means to gather evidence for future randomised controlled trial (RCT) research grant submissions (Lambrechts et al. 2015).

Dengue Vaccines

Significant progress has been made in the search for a tetravalent vaccine, yet the community is still some way off a highly efficacious solution (Halstead 2012). A meta-analysis of vaccine efficacy studies that were conducted during 2012 – 2013 showed that progress is being made, with one vaccine demonstrating 59% efficacy against all DENV serotypes across multiple study populations (da Costa et al. 2014). Further evidence from Thailand published in 2014 showed that among a cohort of 6710 treated individuals, the vaccine achieved efficacy of >65% against DENV 3 and 4. But disappointingly, efficacy against DENV 1 and 2 was only 54.5% and 34.7% respectively (Capeding et al. 2014). Thus, while these results are promising, as yet there is no silver bullet for dengue control. Indeed, recent research indicates that baseline immune status may actually be confounding overall trial results (Dorigatti et al. 2015). Thus more research is needed to ensure that large reductions in dengue incidence are truly indicative of a successful vaccine, rather than reflecting efficacy augmentation through previous immunological challenge from DENV or other flavivirus infections (Dorigatti et al. 2015). And even with the advent of an efficacious vaccine, efforts toward dengue eradication or indeed elimination would almost certainly need to be bolstered with combination vector control strategies, given the unavoidable hurdles of community access, public funding and the need for multiple vaccine doses (Achee et al. 2015).

Predictive Infectious Disease Modelling

Principally, vector-borne disease modelling requires robust and reliable datasets over a large timeframe to smooth out noise associated with short-term variation, and hence produce reliable trends (Reddy 1977). Standard monitoring techniques used for the collection of meteorological data provide a ready source of reliable information, yet these data do not necessarily correspond with the area of interest (Hii et al. 2012a). Also, under-reporting and indeed lag times associated with data capture further confuse epidemiological trends. Any number of variables could be contributing to the problem, from inadequate surveillance to access to health care and/ or patient ambivalence. These symptoms of low-income settings make the creation of forecasting models more difficult than necessary. And yet, the dengue community is desperately seeking methods to define and predict dengue outbreaks.

History of Infectious Disease Modelling

Modelling of vector borne disease transmission was largely pioneered by Ross in 1910 (Ross 1910) and latterly developed by MacDonald in 1957 (MacDonald 1957). The multiple variations of the Ross-MacDonald malaria model has encouraged the use and development of the Entomological Inoculation Rate (EIR) and vectorial capacity, with the concurrent inclusion of standard epidemiological theory such as the basic reproductive number (R0) (Smith et al. 2012). Yet, compared with the century-old modelling history for malaria, dengue modelling still remains in its infancy. Much of the transmission dynamics concepts now applied to dengue were initially developed for yellow fever and transferred to dengue between 1920 and 1980 (Connor & Monroe 1923; Macdonald 1956; Soper 1967; Cornet et al. 1968; Brown 1977; World Health Organisation 1971; World Health Organisation 1972). Subsequently, some of the overarching transmission threshold metrics derived from this process have made their way into the field of dengue, without any evaluative research (Clark et al. 1994; Focks 2004).

Modelling Dengue

Modelling DENV outbreak prediction is hampered by two main factors: poorly evidenced/ defined outbreak definitions and inadequate alarm surveillance systems (Badurdeen et al. 2013; J. Harrington et al. 2013). This is partly due to the complexity and variability of vector borne disease outbreaks (Brady et al. 2015), as models struggle to estimate variable metrics that are central to transmission (Favier et al. 2005). Yet alarm data are routinely captured across many dengue endemic countries (Badurdeen et al. 2013), even if these methodologies require improvement. By identifying fluctuations in such covariate (alarm) data, these variables may demonstrate predictive qualities that warn of forthcoming outbreaks. Consequently, such advance warnings provide epidemiologists and programme managers with temporal and spatial information, as can be used to advise on the deployment of surge capacity clinical preparations and entomological control measures. In so doing, already limited resources can be utilised more efficiently in discrete spatial locations that align with the predictions of the model.

Improvements in modelling dengue transmission dynamics have been forthcoming, with a number of recent papers describing retrospective correlations observed between meteorological and epidemiological variables and subsequent outbreaks, often using comprehensive retrospective datasets (Hii et al. 2009; Hii et al. 2012a; Liu-Helmersson et al. 2014; Phung et al. 2015). Indeed these build on earlier modelling efforts that were able to parameterise for discrete contexts and populations, both of which often exhibit heterogeneity in herd immunity and ultimately transmission dynamics (Focks et al. 1995). Yet, these models tend to focus on small spatial areas, often because coarser resolution approaches are beset by problems of spatial and temporal inconsistencies (Brady et al. 2015). However, this should not deter the pursuit of models that may sensitively predict forthcoming outbreaks, as the benefits are multi-fold, and include reducing the enormous economic burden (Stahl et al. 2013), as well as stemming the manifold increase in dengue incidence that has persisted throughout the 21st Century (Bhatt et al. 2013; Kroeger et al. 2006).

A Confusing Epidemiological Picture

Modelling dengue can be problematic due to the concurrent expanding distribution of the alphavirus, chikungunya (CHIKV). CHIKV has re-emerged as a substantial public health concern (Burt et al. 2012) and now poses a threat to many naïve populations in low-income nations (Burt et al. 2012). Currently, the global burden of CHIKV remains unknown, however, in the Western Hemisphere, the first autochthonous CHIKV case was reported in December 2013, and by August 2014, over half a million suspected or confirmed cases had been recorded throughout the Americas (Staples & Fischer 2014), clearly demonstrating rapid dispersal of this virus. From a modelling perspective, this would not ordinarily be a concern, however clinical symptoms are very similar to dengue and therefore tend to bias dengue incident cases considerably. Modelling techniques should aim to collect coincident CHIKV and DENV cases to ensure that the spread of this virus, and others such as Zika can be accurately controlled for in subsequent analyses.

Systematic Reviews

Systematic reviews are essential to the scientific process and mitigate much of the bias associated with non-systematic literature reviews and expert commentaries (Mulrow 1994; Antman et al. 1992). They provide overarching conclusions on some of the most fundamental scientific questions, in particular, they focus on evaluating intervention effectiveness and the mechanism(s) behind it (Armstrong et al. 2007). They are able to do this by drawing on the current scientific evidence base available in the public domain (Hemingway & Brereton 2009), and synthesising individual published results

(Higgins & Green 2015; Mulrow 1994).

Methodologies

The specific aim of a systematic review is to consolidate and interpret existing scientific evidence, which is done in an equitable, replicable and systematic manner (Higgins & Green 2015). These considerations ensure that the methodologies used can be adequately followed to reproduce the same results, thereby validating the research (Higgins & Green 2015). Important methodological tools that specifically aid this process include the use of Boolean operators amongst free text terms that help unambiguously focus the search area of interest (Higgins & Green 2015). These search constructs shape the search by casting a 'net' around the data of interest. Researchers can either cast a wider net using broader search constructs that will capture any publication distantly related to the question of interest, or use a narrower search construct to immediately focus on a widely researched niche area, thereby excluding many related but ultimately irrelevant articles. Once past this stage, inclusion/ exclusion criteria are used to provide a filter to ensure that the results best represent the area and question of interest, and importantly, include/ exclude certain study designs (Jackson et al. 2004). This approach is particularly useful as it allows researchers to filter large amounts of data based on factors that are not readily searchable using the search constructs described above.

Scientist/ Practitioner Aids

Summarising the available evidence is desirable for both health practitioners and scientists due to the wealth of expanding and evolving information within any given field (Mulrow 1994). Equally, synthesising data from a variety of related, but inherently disconnected studies remains a particularly difficult task (Antman et al. 1992), given that: a) many studies may be missed during the search process; b) the various effect measures between studies may be incomparable; c) study designs will vary (some of which are more robust than others) (Jackson et al. 2004); d) sample sizes vary dramatically and e) population level effects smooth the variation between demographics (Mulrow 1994). These factors tend to confuse the picture, as scientists/ practitioners alike cannot adequately quantify such heterogeneous study characteristics (Jackson et al. 2004) or indeed weight studies accordingly, without the use of appropriate, technical software (Armstrong et al. 2007). Naturally, for these reasons alone, it has become increasingly important that systematic protocols and tools are used to enable the impartial revision and synthesis of existing literature, to arrive at an evidence-based conclusion. After all, these results will inform the next phase of field development and provide overarching recommendations on hugely important issues, ranging from drug/ vaccine efficacy and safety profiles, to user acceptability and feasibility studies.

Introduction to Meta-analyses

Meta-analyses are a method used to improve the power and precision of systematic reviews by amalgamating quantitative data from individual publications (Mulrow 1994). Usually, software (Review Manager (RM)) is used to generate forest plots of measures of effect, calculated either within RM using original summary statistics (mean, standard error, confidence interval), or the available published metrics (Higgins & Green 2015). Importantly, the forest plot utilises comparable outcome measures and is able to weight each study according to the study sample size (Egger et al. 1997). This enables the reader to easily assess the relative impact of any given study, as well as consider the overarching conclusion, drawn from statistically combining the measures of effect from multiple studies into one pooled outcome statistic (Mulrow 1994).

Sensitivity analyses can be used to explore alternative meta-analyses where multiple decisions were made that may have altered the conclusions (Higgins & Green 2015). These provide the reviewer with a systematic method for analysing all those decisions that were based upon non-deliberate or ambiguous decision-making (Higgins & Green 2015). Indeed, it can be argued that ambiguous decisions should be mitigated through protocols at the beginning of the study. While this is taken into account by the use of inclusion/ exclusion criteria that help frame the context, much of the time, questions arise during the process that the reviewer(s) did not foresee at inception (Higgins & Green 2015).

Importance of the process

Standard literature reviews are very familiar to science. They are commonplace in the theses of students, feature in grant applications and are conducted before primary research. They help clarify prevailing trends and possible future goals. Yet, literature reviews are distinctly different from systematic reviews, as the former suffers from many forms of bias and inconsistency. Indeed, they also suffer from the preconceptions of the reviewer. Conversely, as already highlighted, systematic reviews are designed to minimise such bias. For this reason, systematic reviews are much more valuable than their counterparts, in that they are able to both shape and define the current status quo, as well as influence future research avenues. Without them, some important topics would still remain controversial, including: 1) the positive role of statins in cardiovascular disease (Taylor et al. 2013); 2) the effectiveness and safety profile of the MMR vaccine (Demicheli 2014); and 3) the impact of insecticide treated bed nets in reducing malarial childhood morbidity and mortality (Lengeler 2004). Such influential systematic reviews and meta-analyses ensure that science is fairly appraised, relevant, accessible and provides direction for future research for those scientists who contribute to the field and practitioners who rely on it to make informed decisions.

The IDAMS Consortium - International Consortium on Dengue Risk Assessment, Management and Surveillance (www.idams.eu)

Broadly speaking, IDAMS is a EU-funded colloquium of international experts established to improve dengue policy. It is one of three EU consortia funded simultaneously for research on dengue, to develop new and innovative tools to augment existing global dengue control strategies (Jaenisch et al. 2013). The project duration is 5 years, running from 2012 – 2016, and is divided into 6 Work Packages.

This thesis is a product of work wholly within Work Package 3, entitled “Effective, affordable and evidence-based dengue early warning and response systems”. Work Package 3 has a number of broad approaches comprising 1) systematic reviews; 2) country case studies; 3) the evaluation of vector control tools and strategies all leading to the goal of development and evaluation of a predictive dengue outbreak-modelling tool.

Objectives

1. To conduct a systematic review to identify and assess dengue vector surveillance methods as used currently for routine entomological monitoring, and assess the value of the indices used for predicting dengue outbreaks and monitoring the impact of the response.
2. To systematically review the literature for impact of vector control on entomological indices and/ or dengue incidence, and conduct a meta-analysis to analyse and interpret the findings.
3. To undertake a randomised controlled trial to evaluate the impact on vector populations of a range of existing and novel dengue vector control interventions, with specific relevance to outbreak response.
4. Evaluate a retrospective model that assesses the relation between independent alarm variables and dengue incidence in an effort to predict dengue outbreaks.

CHAPTER 2

ASSESSING THE RELATIONSHIP BETWEEN VECTOR INDICES AND DENGUE TRANSMISSION: A SYSTEMATIC REVIEW OF THE EVIDENCE

The results presented in this chapter have been published as the manuscript:

Bowman, L.R., Runge-Ranzinger, S. & McCall, P.J., 2014. Assessing the Relationship between Vector Indices and Dengue Transmission: A Systematic Review of the Evidence. *PLoS Neglected Tropical Diseases*, 8(5), pp.e2848. doi:10.1371/journal.pntd.0002848.

ABSTRACT

Background

Despite doubts about methods used and the association between vector density and dengue transmission, routine sampling of mosquito vector populations is common in dengue-endemic countries worldwide. This study examined the evidence from published studies for the existence of any quantitative relationship between vector indices and dengue cases.

Methodology/Principal Findings

From a total of 1205 papers identified in database searches following Cochrane and PRISMA Group guidelines, 18 were included for review. Eligibility criteria included 3-month study duration and dengue case confirmation by WHO case definition and/or serology.

A range of designs were seen, particularly in spatial sampling and analyses, and all but 3 were classed as weak study designs. Eleven of eighteen studies generated *Stegomyia* indices from combined larval and pupal data. Adult vector data were reported in only three studies. Of thirteen studies that investigated associations between vector indices and dengue cases, 4 reported positive correlations, 4 found no correlation and 5 reported ambiguous or inconclusive associations. Six out of 7 studies that measured Breteau Indices reported dengue transmission at levels below the currently accepted threshold of 5.

Conclusions/Significance

There was little evidence of quantifiable associations between vector indices and dengue transmission that could reliably be used for outbreak prediction. This review highlighted the need for standardized sampling protocols that adequately consider dengue spatial heterogeneity. Recommendations for more appropriately designed studies include: standardized study design to elucidate the relationship between vector abundance and dengue transmission; adult mosquito sampling should be routine; single values of Breteau or other indices are not reliable universal dengue transmission thresholds; better knowledge of vector ecology is required.

INTRODUCTION

Dengue is endemic throughout the tropics, and almost half of the world's population are at risk of infection, 75% of whom live in the Asia-Pacific region (World Health Organisation 2012a). It has been confirmed in 128 countries worldwide (World Health Organisation 2012a; Brady et al. 2012) and can cause major social and economic consequences (Luz et al. 2011; Underagga et al. 2013; Shephard et al. 2013; Halalsa et al. 2012; Martelli et al. 2011). Dengue is transmitted by *Aedes* mosquitoes, primarily by the highly urban-adapted vector *Aedes aegypti*, and a secondary vector *Aedes albopictus* (Lambrechts et al. 2010). *Ae. aegypti* thrives in the man-made urban environment, particularly in deprived communities where water storage is routine, sanitation is poor and non-biodegradable containers accumulate.

The abundance of dengue vector species as well as dengue transmission generally show seasonal variation. Depending on the local ecology, these patterns can be in part driven by meteorological parameters such as rainfall and temperature (Barrera et al. 2011; Campbell et al. 2013). Vector surveillance is recommended by WHO and is a routine practice in many dengue-endemic countries to provide a quantifiable measure of fluctuations in magnitude and geographical distribution of dengue vector populations, ultimately with the purpose of predicting outbreaks and evaluating control (World Health Organisation 2009). The standard protocol relies on the *Stegomyia* indices, which sample the immature mosquito stages (larvae and pupae) alone (Focks 2004). This approach was developed over 90 years ago (Connor & Monroe 1923) for yellow fever, a markedly different infection (zoonotic in origin though ultimately transmitted between humans by *Ae. aegypti*) during a very different era (*i.e.* in terms of urbanization levels and human population densities). Focks (2004) questioned the reliability and sensitivity of the *Stegomyia* indices because they correlate poorly with abundance of adult mosquitoes, (*i.e.* the actual vector stage) which should be sampled directly (Focks 2004). Focks and others recommended sampling adult mosquitoes directly or indirectly via pupal/demographic surveys (calculating a pupae per person/area index, defined as the number of pupae divided by the number of residents/area surveyed) (Focks 2004; Focks & Chadee 1997). Indices based on actual counts of adult female *Ae. aegypti* infesting houses are likely to be the most accurate, but this is rarely done (Focks 2004).

The *Stegomyia* indices remain central to the monitoring of dengue vector populations. The most commonly used indices are the House (or 'premise') index (HI - percentage of houses infested with larvae and/or pupae;) the Container index (CI - percentage of water-holding containers infested with larvae and/or pupae) and the Breteau index (BI - number of positive containers per 100 houses inspected) (World Health Organisation 2009). Variations in sampling protocols are common and can lead to significant variations in indices: *e.g.* sampling may be carried out indoors or outdoors only, or at both locations; the presence of cryptic breeding sites may lead to under-sampling or complete omission of certain sites; failure to distinguish *Aedes aegypti/albopictus* from other common mosquito species, or from each other, may lead to overestimates. Little is known about the relationship between differing proportions of the various sampled larval instars and the accuracy of these data as proxy measures of adult mosquito abundance (Focks & Chadee 1997). Finally, although ovitraps (water-filled pots in which *Aedes aegypti* lay their eggs) are widely used as a simple sampling tool, Focks (Focks 2004) showed very convincingly that their reliability is limited to indicating vector presence or absence.

Despite these doubts, many dengue control authorities worldwide routinely collect vector population data based on these indices, although the mathematical relationship between any of the indices and dengue transmission is far from clear. Indeed, thresholds indicating dengue outbreak risk for House and the Breteau indices (HI = 1%, BI = 5) have been used for many years (Tun-Lin 1996; Kuno 1995), even though these values were developed for yellow fever many decades earlier. Simple thresholds may be valid in some situations (Sanchez et al. 2010), but a universal critical threshold applicable across many contexts, has never been determined for dengue. In pursuing the goal of identifying dengue thresholds, Scott & Morrison (Scott & Morrison 2003) defined the fundamental knowledge gaps as: 1) what is an acceptable level of dengue risk?; 2) what are the mosquito densities necessary to achieve that goal?; 3) what is the best way to measure entomological risk?; 4) at what geographic scale are the components of dengue transmission important? While a number of mathematical models have explored the value of thresholds or rates of change in the vector population for the prediction of dengue outbreaks (Focks et al. 2001; Ellis et al. 2011), these knowledge gaps remain and continue to hinder progress (Andraud et al. 2012). For convenience, dengue outbreaks are often defined as periods when dengue incidence is equivalent to the mean plus 2 standard deviations during the same month of the previous year (Badurdeen et al. 2013).

Effective dengue surveillance and early warning systems, using information from multiple epidemiological sources, are an important goal for numerous countries worldwide. To determine the value of vector surveillance for such systems, the findings of a systematic review examining the evidence for a relationship between mosquito indices and dengue cases are reported here.

METHODS

Objectives

The aim of the study was to evaluate the potential value of vector or entomological survey data for dengue surveillance by examining the evidence from studies that investigated quantitatively the relationship between vector indices and dengue cases. The specific objectives were:

1. To identify vector surveillance methods and indices used for the routine monitoring of *Aedes aegypti* or *Aedes albopictus* populations in any geographic location.
2. To examine how entomological indices correlated with dengue incidence.
3. To examine the effectiveness or accuracy of vector surveillance in predicting dengue outbreaks and consider how this might be improved.

Search Strategy

A review protocol was established and agreed upon by all authors. Guidelines from the Cochrane Handbook for Systematic Reviews and the PRISMA Group were followed as standard methodologies (Centre for Reviews and Dissemination 2009; Moher et al.

2009). The databases WHOLIS, PubMed, EMBASE, LILACS and Web of Science were searched using the Medical Subject Heading (MeSH) "dengue" followed by the Boolean operator "and" combined with one of each of the following 'free text' terms in succession: 'entomological surveillance', 'oviposition trap', 'house index', 'container index', 'Breteau index', 'pupal index', 'pupal survey', 'adult collection', 'sticky trap', 'aspirator collection', 'resting collection', 'landing collection', 'vector density'. The reference list of each of the included studies was also searched, and "grey literature" was sought by communication with authors for cited unpublished documents.

Results were collated in EndNote (EndNote X5, Build 7473) where abstracts were reviewed in accordance with agreed inclusion and exclusion criteria. Full text review was completed using 'Papers' (Papers 2, version 2.2.10). No limits were placed on year of publication, language or location.

Inclusion and Exclusion Criteria

The criteria for inclusion or exclusion of individual studies were set in advance (Table 2.1) and were used to assess each abstract and/or the full text.

Table 2.1. Criteria for inclusion or exclusion of studies. Criteria used to assess each study at each stage throughout the review.

Inclusion Criteria	**Exclusion Criteria**
Any study where entomological surveillance of *Aedes* spp. was undertaken for >3 months (or for the duration of a dengue outbreak) in conjunction with number of reported dengue cases	Studies with only one outcome of interest (entomological surveillance OR dengue cases);
Any study type with all empirical data gathered within the same time period	Opinion papers; review articles; retrospective analyses comparing data generated at different time points
Confirmed and/or probable dengue cases identified using WHO standard case definition and/or serology	Qualitative dengue reports

Definitions

The following definition was used for the term 'vector surveillance': "Any on-going surveillance of entomological indices, including larval indices (House Index (HI), Container Index (CI), Breteau Index (BI)), pupal indices (Pupal Productivity Index (PPI) and other variations), oviposition trap data and data from adult mosquito collections (methods include sticky, traps, CO_2, odour-baited, visual or other traps, resting catches, human landing catches), used in relation to dengue outbreak/control."

Quality assessment

Given the strict nature of the inclusion criteria, study design was assessed at the data extraction stage using the Quality Assessment Tool for Quantitative Studies (QATQS) (National Collaborating Centre for Methods and Tools 2008). QATQS provides a recognized standardized method to assess study quality by assigning scores based on possible selection bias, study design, confounders, data collection methods, intervention integrity and statistical analyses. This ensured each study could be ranked qualitatively. The study design classes were intervention, case-control and longitudinal. If clarification was required, authors were contacted for any missing data or information.

Data extraction and assessment

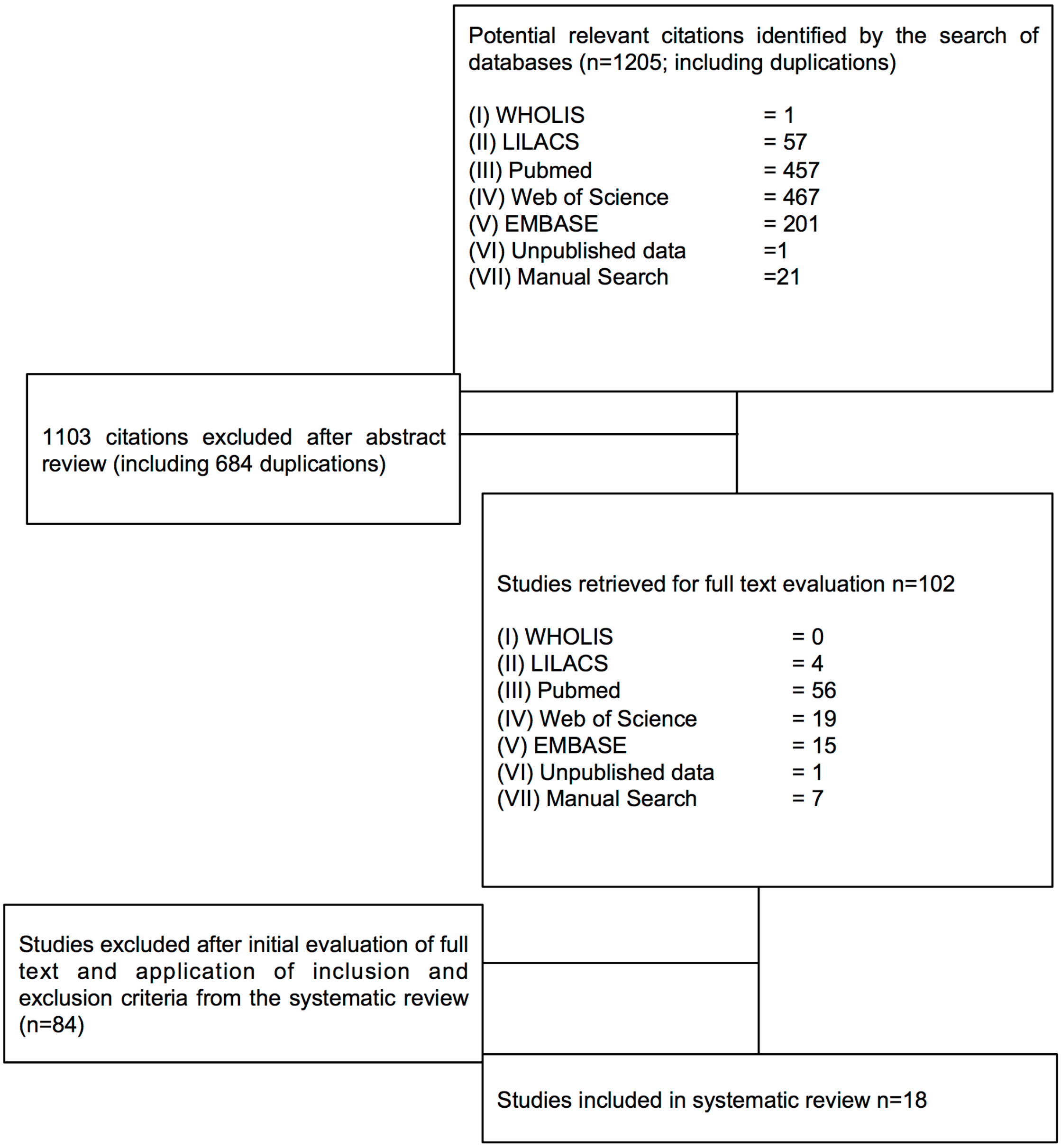
The information extracted included first author, year of publication, year of study, population size, study design, indices and case definitions, study objectives, duration of study, frequency of data collection, results and conclusions (as viewed by all reviewers; Appendix 1). A table of bias was created to help identify the strengths and weaknesses of each study (Appendix 2).

RESULTS

A total of 1205 potentially relevant studies were identified in the database search. After reviewing abstracts, 102 were selected and retrieved for full text evaluation, of which 18 were considered to have satisfied all inclusion and exclusion criteria and explored in detail (Figure 2.1) (1-18).

Figure 2.1. Search tree.

Diagram of searches performed and the number of articles returned and examined at each stage.



Regarding the 84 studies excluded, the most common reasons for exclusion were: study duration less than 3 months (22 studies); absence of a reliable dengue case definition (21 studies); use of datasets that did not correspond temporally or spatially (19 studies). Note that although such dislocated spatial comparisons were not captured by the exclusion criteria originally defined (simply because it had not been expected), exclusion at this point was considered to be valid. Other reasons for exclusion were: measurement of only one outcome (*i.e.* vector or dengue cases only: 9 studies); opinion or review articles (8 studies); use of incomplete datasets – where only 'selected' portions of all of the data available during the study period were used (5 studies). Again, although the latter reason was not captured by the original criteria, exclusion of studies where this occurred was considered to be valid. Full details of the 18 studies reviewed are summarised in the supporting data files (Appendix 1, Appendix 2).

The origin of the data used in analyses differed between studies. Some generated novel data as an integral part of the study, thus ensuring complete or independent control over the quality of the data obtained, while others obtained existing or retrospective data from external sources, including local surveillance data (*e.g.* local government records, private companies, hospitals or health centres, independent physicians and self-reported data). Twelve studies generated vector data (3-5,7-13,15-17), five generated dengue case data (2,3,8,9,11), four of which generated both vector and dengue case data (3,8,9,11).

Study Design

Fourteen studies were longitudinal, two were case-control, one was an ecological study (as defined by the unit of analysis) and one was a vector control intervention. Applying QATQS (National Collaborating Centre for Methods and Tools 2008), fifteen studies (1,3-6,8-14,16-18) scored 3 (defined as a weak study), two studies (2,15) scored 2 (a moderate study design) and one study (7) scored 1 (a strong study design) (Appendix 2). In the latter study, Chadee and colleagues (7) used controls matched on age and sex from a neighbouring community, although the report did not state whether or not this process was randomized.

Vector sampling

Details of the sampling protocols used in each study are shown in Table 2.2. Eleven of eighteen studies generated indices for immature stages of the vector by collecting combined larval and pupal numbers to calculate either the CI, HI or BI (1-5,7,8,10,13-15. One of these (10) combined *Ae. aegypti* and *Ae. albopictus* data. Four studies sampled only larvae (6,9,17,18).

Table 2.2. Details of vector sampling methods used and correlation of vector indices with dengue transmission in the studies reviewed.

All studies reported *Ae. aegypti* alone unless indicated otherwise. HI = House Index (% houses with larvae and/or pupae); CI = Container index (% water-holding containers with larvae or pupae); BI = Breteau index (no. positive containers per 100 houses inspected); BI_{max} is defined as the highest or 'maximum' block level BI in a neighbourhood; Pupal index = pupae per person/premise defined as no. pupae divided by the number of residents/ premises.

Immature vector samples are denoted as: ◆ larvae only; ■ larvae and pupae; ⊙ *Aedes aegypti* & *Aedes albopictus* combined.

For the column Adult Mosquitoes Sampled, cells marked +/- are both positive and negative; + is positive; - is negative; ~ is unknown.

Cells marked ✓ indicate the sampling activity was done.

The sample spatial unit referred to as '**Premise***' is the 'premise with cardinal points index' [34], which utilises the points of a compass to select households;

'N'hood' = neighbourhood.

In the right-hand column, the reported association between vector indices and dengue cases is classed as: '**+**' positive association; '**-**' no association; '**+ -**' ambiguous association; '**~**' inconclusive or weak association.

Absence of any entries in cells indicates no data or information was reported

Ref. Number	Study	Immature Vector Indices					Adult mosquitoes sampled	Egg (ovitrap) sampled	Location		Sample spatial unit	Significant ($p \leq 0.05$) increase in vector indices recorded during dengue transmission
		CI	HI	BI	BI_{max}	Pupal Index			Indoor	Indoor + Outdoor		
1	Sanchez *et al.*, 2010.			■	■				✓		Block; N'hood	~
2	Sanchez *et al.*, 2006.	■	■	■	■				✓		Block; N'hood	+
3	Chadee, 2009	■	■	■		pupae/ person				✓	Premise	+
4	Pham *et al.*, 2011	■	■	■					✓		Premise	+
5	Gurtler *et al.*, 2009		■	■					✓		N'hood; City	~
6	Katyal *et al.*, 2003	◆	◆	◆								~
7	Chadee *et al.*, 2005			■						✓	Premise*	+/-
8	Romero-Vivas & Falconar, 2005	■	■	■		pupae/ premise				✓	Premise	-
9	Foo *et al.*, 1985		◆	◆					✓		Premise	-
10	Sulaiman *et al.*, 1996		■⊙	■⊙					✓		City zone	+/-
11	Honorio *et al.*, 2009						✓	✓			Premise	-
12	Rubio-Palis *et al.*, 2011						✓		✓		Premise	+
13	Lin & Wen, 2011			■	■					✓	District; Min admin unit	+/-
14	Chaikoolvatana *et al.*, 2007	■	■	■						✓	Village	~
15	Chadee *et al.*, 2007		■	■						✓	County	~
16	Correa *et al.*, 2005						✓				District; Trial area	+/-
17	Fernandez *et al.*, 2005	◆	◆	◆							Premise	+/-
18	Arboleda *et al.*, 2012			◆							0.25 km^2	-

Thirteen studies reported the location of the immature stage mosquito samples: six studies sampled both indoor and outdoor containers (3,7,8,13-15), while seven searched indoor containers only (1,2,4,5,9,10,12). Thus, where reported, all studies included indoor sampling.

Pupal indices were reported in two studies (3,8). Adult mosquitoes were sampled in three studies (11,12,16).

Relationship between entomological indices and dengue cases

Thirteen studies examined the association between entomological indices and dengue, using a range of different statistical approaches. Seven studies calculated regression coefficients (9,10,12,13,16-18), two calculated rate ratios (4,11), one calculated odds ratios (2) and two calculated the G-test for significance (5,9). One study used only specificity, sensitivity and positive and negative predictive values (1).

The spatial unit of analysis, an important consideration in dengue epidemiology (see Discussion) varied considerably across studies, with units ranging from individual houses, housing blocks and clusters to neighbourhoods and even large municipalities (Table 2.2).

Four studies reported statistically significant positive relationships between entomological indices and dengue incidence (2-4,12). Of these, only one sampled adult mosquitoes (33% of those studies that sampled adults) (12) while the remainder sampled immature stage mosquitoes (20% of all those that sampled immatures) (2-4) (Table 2.2). These are discussed in detail here.

Evidence for positive correlation between vector indices and dengue cases

Sanchez (2006) (2) conducted a case control study using two geographical units for analysis, blocks (units of approximately 50 houses) and neighbourhoods (each containing approximately 9 blocks). Any block or neighbourhood with at least 1 confirmed case was considered positive, while a control was defined as a block or neighbourhood without confirmed cases. HI and BI mean values were "consistently, substantially and significantly higher" in blocks with dengue cases compared with control units. An odds ratio (OR) of 3.49 ($p<0.05$) for dengue transmission was associated with the presence of a single positive container in a block; fifteen of the seventeen dengue cases recorded lived in a neighbourhood where at least 1 block had a BI > 4.

In Trinidad, Chadee (2009) (3) compared retrospective routine entomological household data with concurrent entomological data taken from confirmed dengue households, using a cardinal points approach (*i.e.* the 'index' house plus the four adjacent houses at its cardinal points). Chadee found that significantly more ($P < 0.001$) immatures were collected during dengue case investigations than during the routine inspection and treatment cycles. The report also stated that pupae per person indices were higher and significantly more adults emerged (as a function of total pupae count collected from household containers) at locations where dengue was confirmed at the index house, compared with routine investigations.

Pham *et al.* (2011) (4) examined monthly dengue case data, vector larval indices and meteorological data from central Vietnam, between 2004 and 2008. They found significant associations between all entomological indices and dengue cases by univariate analysis but only the HI and "household mosquito index" (not defined in the paper), temperature and rainfall were significant after multivariate analysis.

In Venezuela, Rubio-Palis et al. (2011) (12) used a simple regression analysis to investigate correlations between vector indices, climatic variables and dengue incidence for the period 1997-2005. Analyses indicated a significant relationship (R^2=0.9369) between the numbers of dengue cases, *Ae. aegypti* abundance (both immatures and adults) and rainfall, using the regression equation: Dengue = 3343.36-0.88098*total mosquitoes+60.4212 Aedes/house-99.7139+1.38476*Maximum temperature*precipitation.

Acknowledging the retrospective nature of the study, the authors expressed caution in the explanatory value of the findings. Moreover, another limitation was that entomological data were derived only from actual homes and neighbouring houses of confirmed dengue cases but no data were collected from 'control' houses.

Value of vector indices for advance warning of dengue outbreaks

Within these four studies was some additional evidence that observed changes in vector indices might be useful for the prediction of impending dengue transmission or outbreaks. In Cuba, Sanchez (2006) (2) reported that blocks with BI_{max} (defined as the highest or 'maximum' block level BI in a neighbourhood) values greater than 4 were significantly more likely to record positive cases in the following month, and had a 3-5 times greater dengue risk in comparison with control blocks. The report concluded that $BI_{max}>4$ and neighbourhood BI>1 during the preceding 2 months provided "good predictive discrimination". In northern Venezuela Rubio-Palis *et al.* (2011) (12) found the most significant correlation between rainfall levels and the appearance of dengue cases two months later, indicating that the magnitude of outbreaks might be predictable to some extent following periods of rainfall. Pham *et al.* (2011) (4) confirmed an association between dengue transmission and periods of higher rainfall and mosquito abundance in the central highlands of Vietnam, but did not indicate whether this could be used in advance of transmission as a predictive tool.

Unreliable or absence of correlation between vector indices and dengue cases

A further five studies (7,10,13,16,17) reported ambiguous evidence of associations, both positive and negative, between entomological data and dengue cases. In Belo Horizonte, Correa *et al.* (2005) (16) found a 5 -7 fold increase in mean monthly dengue incidence where the 'infestation rate' (defined as house index) was "between 1.33% and 2.76% and equal to or higher than 2.77% when compared to areas showing 0.45% or less", although it was unclear whether or not this was statistically significant. They reported a weak and insignificant correlation between HI and dengue cases (R=0.25 (p=0.41) at the municipal level, and weak significant correlations at the district level (R=0.21; p=0.02) and village level R=0.14 (p=0.00). Sulaiman *et al.* (1996) (10) reported a significant correlation between BI and HI and dengue cases in certain areas of Kuala Lumpur, but not in others. In Trinidad, Chadee *et al.* (2005) (7) found that 75% of DHF cases were located in areas where BI was greater than 10, although BI and dengue infections were rarely correlated. An additional two studies reported either very low correlations between vector indices and dengue (17), or utilized highly variable inter-annual data precluding such analyses (13).

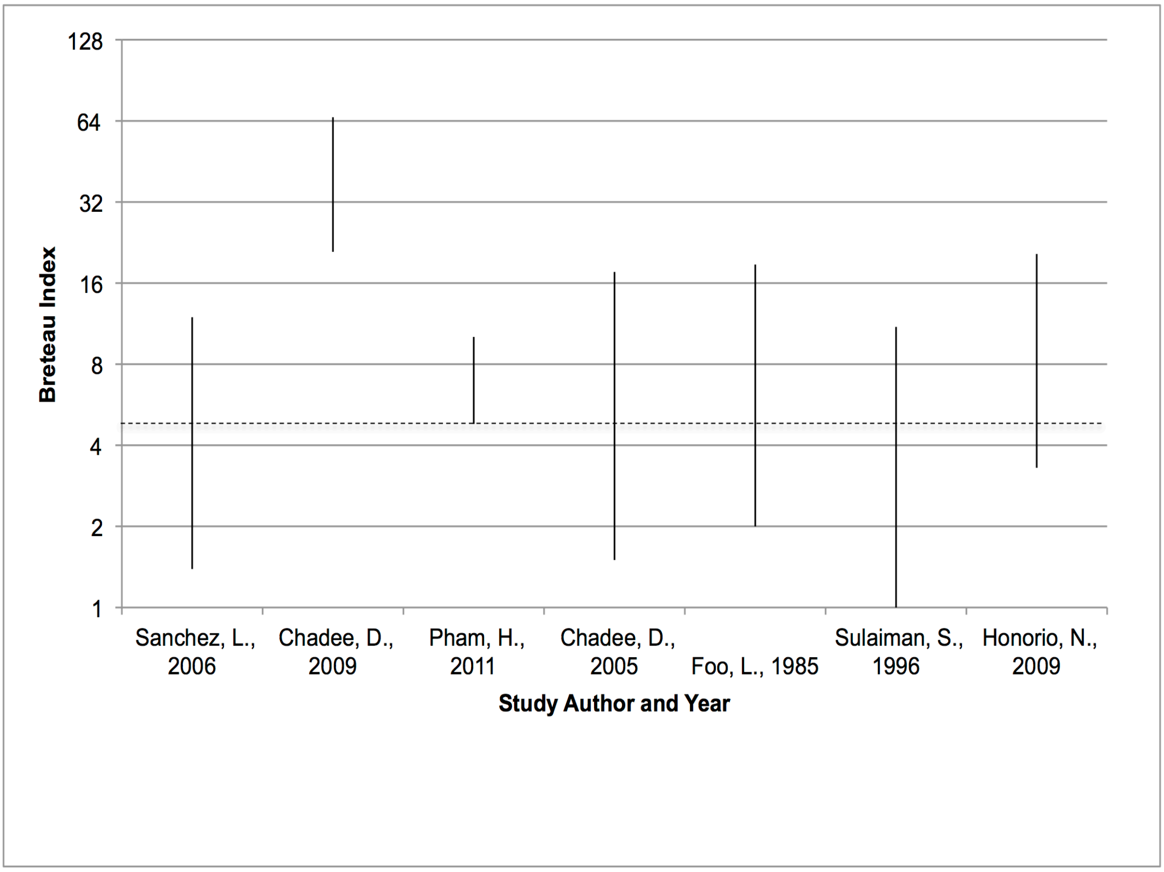
Four studies, from Malaysia (9), Brazil (8) and Colombia (8,18) found no statistically significant relationships between entomological indices and dengue cases. Foo *et al.* (1985) (9) observed a positive but non-significant association between dengue cases and HI and BI, which they suggested might have been influenced by the small sample size, the presence of *Ae. albopictus* and socio-demographic factors. Honorio *et al.* (2009) (11) found no significant associations between recent dengue cases and *Ae. aegypti* densities and proposed that infections received outside the home were responsible. In Colombia, Romero-Vivas and Falconar (2005) (8) reported distinct positive temporal correlations between the larval density index and pupal density index ($p<0.005$) and a negative association between the larval density index and egg density index ($p<0.01$); however, they found no correlation between any of the larval, pupal or adult indices with either rainfall or dengue-like cases. The spatial model of Arboleda *et al.* found no indication that the BI was in any way correlated with the dengue cases or those areas predicted as 'suitable' (18).

In the remaining studies (1,5,6,14,15) a variety of mixed, inconclusive or weak associations were reported. Gurtler *et al.* (2005) conducted analyses on the effect of a given intervention on mosquito indices but not on dengue cases (5). Although Katyal *et al.* (2003) (6) did not present any statistical analyses, they reported the observation that over a five-year period, a fall in cases was visually correlated with a fall in indices. However, they conceded that "an increasing trend of cases was observed [in 2001] in spite of a further declining HI trend", and concluded that HI had no predictive value at the 'macro' level. Despite the absence of statistical analysis, Chaikoolvatana *et al.* (2007) (14) reported a suggestive link between dengue haemorrhagic fever (DHF) during peak annual rainfall months and high abundance of mosquitoes. Chadee *et al.* (2005) observed ambiguous associations, with BI partially correlating with dengue fever cases for two out of three years (15). As in their earlier study at the same Cuban location (2), Sanchez et al (1) reported that while $BI_{max}\geq4$ was a useful predictor for outbreaks at the block level, sensitivity during outbreaks ranged between 62% and 81.8% and specificity between 71.9% and 78.1%.

Use of vector indices as transmission thresholds

The Breteau Index (BI) was used as an outcome measure in seven studies (2-4,7,9-11) and BI_{max} threshold was considered in three (Table 2.2) (1,2,13). Here, BI values ranged from 1 to 66 during periods when dengue transmission was recorded (Figure 2.2). In other studies, both recent (Hanna et al. 2007) and historic (MacDonald 1956), dengue transmission was recorded when BI values were lower than the widely accepted transmission threshold of 5. Notably, in a study in Trinidad, 'high' transmission (25-40 cases for 75% of sample 'cycles') took place in areas with relatively 'low' abundance (~BI<5) while, conversely, a consistently higher BI of 5.4 in neighbouring areas did not result in dengue cases (7). In Rio de Janeiro, the BI did not correlate with dengue incidence and transmission occurred in association with a wide range of BI levels (range 3.30 – 20.51) (11).

Figure 2.2. Range of Breteau indexes reported during dengue transmission.



Dotted line indicates a BI value of 5, which has been considered a transmission threshold for dengue (Scott & Morrison 2003; (18), Kroeger et al. 2006). Note: Includes all data where available, whether statistically significant or insignificant.

DISCUSSION

With worldwide dengue transmission levels at an all time high, predicting dengue outbreaks in advance of their occurrence or identifying specific locations where outbreak risks are highest is of critical importance. This review considered the evidence that changes in vector populations can be correlated with dengue virus transmission and whether or not monitoring fluctuations in vector indices might be employed to provide reliable advance warning of impending dengue outbreaks.

Eighteen studies that had the potential to provide evidence of any association between vector indices and dengue incidence were identified and examined. Notably, only 4 studies utilized new data on both vector indices and dengue cases collected *de novo* as an integral part of the study. More common was a reliance on local government-level records for the dengue case data, a practice that potentially introduces error or bias for number of reasons. First, hospital reports are prone to selection bias, as asymptomatic/ inapparent infections may not be recorded and the actual number of cases may have been significantly underreported. Second, there can be a considerable delay between the times of onset of infection and reporting which, if the infection date is not calculated, would result in a temporal mismatch of vector and case data. Third, differences between the geographic location of the vector and dengue case data, or between the spatial units from which each was originally calculated, would result in a geographic mismatch or mask potential relationships, respectively.

The latter point is of particular significance not only from the point of view of these studies, but also when considering the design of future investigations. A growing body of evidence indicates that the distribution of dengue cases typically is highly clustered in both time and space. In various studies, post-dating those reviewed, the size of such clusters ranged from 800m (Vazquez-Prokopec et al. 2010) to less than 100m (Yoon et al. 2012). The effective area of such key 'pockets' or 'hotspots' is likely to be determined by dispersal of the vector (Yoon et al. 2012; Schafrick et al. 2013) which itself can vary over time (Duncombe et al. 2013), and is influenced by house density (Kroeger et al. 2006) and by human movement within and beyond the infection cluster (Stoddard et al. 2009). Consequently, in studies attempting to correlate vector indices with dengue transmission, and where the geographical unit is too large, high vector densities in key dengue hotspots might be diluted by inclusion of neighbouring areas with low densities, thus masking any true relationships [see (38)].

Indeed, human movement potentially confounds dengue vector data that derive from residential areas alone as increasingly, evidence indicates that only a proportion of dengue infections are transmitted in the individual's own home, with many infections (possibly the majority) resulting from bites by virus-infected mosquitoes at other houses, schools, workplaces or numerous locations remote from the home (Stoddard et al. 2013; Stoddard et al. 2013). Clearly, this presents a serious challenge when considering the use of vector data for surveillance and highlights a need for inclusion of data from public locations (Morrison et al. 2006) in addition to residential areas, in any surveillance program.

Returning to the studies examined in this review, the fact that there was no clear indication of any consistent association between vector indices and dengue cases is not unexpected, given the diverse and mostly weak study designs. One study found there was no apparent increase in vector indices coinciding with what was the largest increase in dengue fever cases of all areas studied (13), while in another, dengue transmission remained low despite exceptionally high vector indices (17). In studies where correlations were calculated for HI, BI and dengue cases, regression coefficients ranged from weak/moderate non-significant ($R=0.43$ and $R=0.35$ respectively; $p>0.05$)[38], to moderate significant associations ($R=0.61$ and $R=0.60$ respectively, but only in the urban centre; $p<0.05$) (10).

Only two studies calculated pupal indices, even though fifteen of the eighteen studies reviewed were published more than three years after WHO acknowledged that the traditional *Stegomyia* indices were inadequate for the measurement of dengue vector abundance (World Health Organisation 2000). In the two studies included in this review that calculated pupal indices, only one reported increases in the pupal index, but its relationship with dengue cases was not statistically significant, possibly due to the low numbers of pupae recorded (3,8). A major problem with pupal surveys is the difficulty in locating breeding sites and the potential existence of important or key but cryptic breeding sites (*e.g.* overhead tanks on houses or underground water reserves such as sewers or wells) that may harbour significant proportions of the vector population (Barrera et al. 2008; Pilger et al. 2008).

Clearly, calculation of adult female *Aedes aegypti* indices is the most direct measure of exposure to dengue transmission (Focks 2004). Of the four studies reviewed that reported some correlation between vector indices and dengue cases, two (4,12) recorded adult vector data. The adult population of *Aedes aegypti* is rarely sampled, partly due to the erroneous but commonly held belief that carrying out such sampling is time-consuming, difficult or expensive (Anders & Hay 2012).

Sampling adult female *Aedes aegypti* is a relatively simple task, though it can be limited by the fact that mosquito numbers often remain low during outbreaks (Goh 1997). Nonetheless, it is possible to aim to sample adult mosquitoes as a routine procedure with minimal additional training and resources. A number of novel sampling devices (Maciel-de-Freitas et al. 2008; Barrera et al 2013; Ritchie et al. 2014) offer the potential to monitor vectors during outbreaks (Ritchie et al. 2013) and at the spatial scale required to accurately sample populations of *Ae. aegypti* (Barrera 2011). Simple affordable low-tech tools that enable localized sampling of adult *Ae. aegypti* and other mosquito vectors are available, with initial studies demonstrating their ease and effectiveness in comparison with older methods (Mai et al. 2011; Vazquez-Prokopec 2009). In Brazil, routine sampling of *Ae. aegypti* adults with gravid traps deployed at relatively low densities was used to identify high-risk localities which were then targeted for vector control [68,69]. This 'Intelligent Dengue Monitoring' system was reported to have prevented over 27,000 dengue cases over two 'dengue seasons' between 2009 and 2011 with considerable reductions in cost burden to the communities where it was deployed (Mammen et al. 2008).

None of the studies reported on viral infection rates in the vector. This perhaps is not surprising given that techniques suitable for application in routine surveillance, such as PCR or NS1, have not been available until recently, that vector infection rates with dengue virus are of the order of 1% even in areas where transmission is on-going (Ritchie et al. 2013; Mammen et al. 2008; Garcia-Rejon et al. 2008; Yoon et al. 2012) and the cost of running the large numbers of tests to detect meaningful infection levels could be considered prohibitive for many authorities. Nonetheless, routine screening for dengue virus of trapped adult female *Aedes aegypti* is possible and has been incorporated into the routine surveillance program in Belo Horizonte, Brazil (Figueiredo et al. 2013). The relatively low dispersal rates of *Ae. aegypti* as compared with the high mobility of humans as they commute daily from the home to the workplace, school, etc., means that virus-infection rates in the vector potentially could provide an accurate or epidemiologically valid indicator of dengue risk in any particular locality, thus informing vector control. Clearly, elucidating the relative value of such an index would require substantial research investment, while integrating it into routine surveillance programmes would demand significant sustained investment, but the importance of metrics like the sporozoite or entomological inoculation rates used in malaria epidemiology (Anders & Hay 2012) already indicate the potential.

This review has also demonstrated the unreliability of accepted vector thresholds for dengue transmission. A number of studies reported dengue transmission at BI levels below the currently accepted threshold of 5 (Figure 2.2) (2,7,9-11) or when the HI was below 1% (Goh et al. 1987; Koh et al. 2008). Elsewhere, Focks proposed a pupal productivity index of 0.25 as a threshold for dengue transmission in Honduras (Focks et al. 1995), yet in Brazil dengue transmission occurred at PPI levels of 0.15 (Pilger et al. 2011). While the desire for a single globally applicable transmission threshold is understandable, it seems unlikely that such a threshold exists, given the variety and complexity of other parameters that potentially influence the risk of outbreaks today (Kun 1995; Reich et al. 2013; Rabaa et al. 2013). Chadee concluded in 2009 that dengue transmission occurs, not at a fixed entomologic figure/quantity but rather at a variable level based on numerous factors including seroprevalence, mosquito density and climate (Chadee 2009). It is becoming increasingly apparent that thresholds differ at different locations and in different contexts, and while they must be calculated independently at each location (Kuno 1995; Sommerfeld & Kroeger 2012). Moreover, empirically defining thresholds, which must be expected to be dynamic, rising and falling as the susceptibility of the local population changes, will require comprehensive prospective, longitudinal vector studies (Morrison et al. 2004), with simultaneous monitoring of the relationship between *Ae. aegypti* population densities and dengue virus transmission in a spatially relevant human cohort.

LIMITATIONS

Study Limitations

In spite of reference searches and use of grey literature, publication bias will likely remain given the very nature of a systematic review. However, we also sought to further limit the effect of bias by placing no restriction on language, and those languages encountered were: English, French, Portuguese, Spanish and Chinese.

Additionally, one should be cautious when interpreting these data due to the study design of the 18 articles. As defined by QATAS assessment methods, study design was often weak (15 studies), meaning that studies were prone to bias and confounding factors, which may have skewed some of the reported associations. In addition, most (n=13) studies relied on dengue case data from external sources, rather than obtaining study-generated data. With the exception of vector sampling and generation of vector index, there were few similarities in the approaches across the different studies.

CONCLUSIONS AND RECOMMENDATIONS

Despite the widespread practice of collecting vector population data, the review has revealed that very few rigorous studies have been undertaken to determine the relationship between vector abundance and dengue transmission; of those that have been published, few provide tangible evidence of such a relationship, and therefore it is not possible to draw a firm conclusion. After decades of vector surveillance in many countries and considering the magnitude of the dengue threat today both in those and other countries that have recently experienced major dengue outbreaks, this is disappointing. Yet it is also indicative of the lack of basic knowledge of dengue epidemiology, in particular with regard to transmission, and poorly implemented surveillance methodologies. Clearly, these are major knowledge gaps that require attention with a degree of urgency and the following research priorities are recommended:

- The relationship between vector population abundance and dengue transmission remains unknown and should be quantified. Studies should aim to collect new vector and clinical datasets carefully matched temporally and spatially. Given that epidemiology will vary considerably between different contexts and geographical localities, multiple locations should be investigated.
- The ideal and most powerful approach would be for a series of coordinated studies, to be carried out in multiple locations worldwide, as exemplified by recent examples (Morrison et al. 2004). To facilitate such studies, and ensure higher power in individual and combined datasets, the development of a standardized study design and protocols is a priority.
- Independent spatial entities (districts) are also strongly encouraged to investigate the relationship independently. Many dengue-affected areas (cities, districts or similar spatial units) are likely to have substantial historic vector and dengue data that potentially may be suitable for appropriate analysis.
- Spatial heterogeneity and transmission at sites other than the home must be considered and carefully incorporated into any study design.
- The utilization of single global values of the Breteau (BI) or other vector indices as thresholds for dengue transmission is unreliable and is not recommended.
- While the need for a standardized reliable definition of a dengue outbreak has already been stated elsewhere (Runge-Razinger et al. 2008), research into the relationship between vector abundance and dengue transmission should endeavour to develop a similar approach to defining reliable locality-specific vector population indices (*e.g.* thresholds, rates of increase, etc.) for use as early warning signals for impending increases in dengue transmission.
- Adoption of adult dengue vector sampling by all vector surveillance programs is urged. Various new trapping methods, as well as a simple resting catch approach,

should be evaluated.

- Relationship between larval, pupal and adult stages of the vector population and the factors influencing adult emergence rates remain poorly understood. The paucity of fundamental knowledge of the ecology of mosquito vectors generally and the need for basic studies has been advocated elsewhere (Ferguson et al. 2010; Godfray 2013) and is true for *Ae. aegypti* and *Ae. albopictus*. A greater understanding of the ecology of dengue vectors is essential.

In the absence of definitive evidence that dengue vector surveillance data can contribute to the prediction of dengue outbreaks, it might be tempting to consider abandoning the practice altogether. However, this would be a rash and premature judgment. At the very least, this systematic review has demonstrated that the potential of vector surveillance data has not yet been evaluated. Indeed, its full potential will not be apparent until its contribution to a complete predictive model incorporating all other covariates influencing dengue epidemiology have been considered. That will not be possible until multiple high quality studies investigating the relationship between vector populations and dengue transmission have been carried out.

Included Study References

1. Sanchez, L. et al., 2010. Breteau index threshold levels indicating risk for dengue transmission in areas with low *Aedes* infestation. *Tropical Medicine & International Health,* 15, pp.173-175.
2. Sanchez, L. et al. 2006. *Aedes aegypti* larval indices and risk for dengue epidemics. *Emerging Infectious Diseases*, 12, pp.800-806.
3. Chadee, D.D. 2009. Dengue cases an*d Aedes aegypti* indices in Trinidad, West Indies. *Acta Tropica*, 112, pp.174-180. doi:10.1016/j.actatropica.2009.07.017.
4. Pham, H.V. et al., 2011. Ecological factors associated with dengue fever in a central highlands province, Vietnam. *BMC Infectious Diseases*, 11, pp.172.
5. Gurtler, R.E., Garelli, F.M. & Coto, H.D., 2009. Effects of a five-year citywide intervention program to control *Aedes aegypti* and prevent dengue outbreaks in northern Argentina. *PLoS Neglected Tropical Diseases*, 3, pp.e427.
6. Katyal, R. et al., 2003. Impact of intervention measures on DF/DHF cases and *Aedes aegypti* indices in Delhi, India: An update, 2001. *Dengue Bulletin*, 27, pp.163–167.
7. Chadee, D.D., Williams, F.L. & Kitron, U.D., 2005. Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998. *Tropical Medicine & International Health*, 10, pp.748-754.
8. Romero-Vivas, C.M.E. & Falconar, A.K.I., 2005. Investigation of relationships between *Aedes aegyp*ti egg, larvae, pupae, and adult density indices where their main breeding sites were located indoors. *Journal of American Mosquito Control Association*, 21, pp.15-21.
9. Foo, L.C. et al., 1985. Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *South East Asian Journal of Tropical Medicine and Public Health,* 16, pp.560–568.
10. Sulaiman, S. et al., 1996. Relationship between Breteau and House indices and cases of dengue/dengue hemorrhagic fever in Kuala Lumpur, Malaysia. *Journal of American Mosquito Control Association*, 12, pp.494-496.
11. Honorio, N.A. et al., 2009. Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil. *PLoS Neglected Tropical Diseases*, 3(11), pp.e545.
12. Rubio-Palis, Y. et al., 2011. Influence of climatic variables on dengue cases and abundance of *Aedes aegypti* (Diptera: Culicidae) in Maracay, Venezuela. *Boletin de Malariologia y Salud Ambiental,* 51, pp.145-157.
13. Lin, C.H. & Wen, T.H., 2011. Using geographically weighted regression (GWR) to explore spatial varying relationships of immature mosquitoes and human densities with the incidence of dengue. *International Journal of Environmental Research & Public Health*, 8, pp.2798-2815.
14. Chaikoolvatana, A., Singhasivanon, P. & Haddawy, P., 2007. Utilization of a geographical information system for surveillance of *Aedes aegypti* and dengue haemorrhagic fever in north-eastern Thailand. *Dengue Bulletin*, 31, pp.75-82.

15. Chadee, D.D. et al., 2007. Climate, mosquito indices and the epidemiology of dengue fever in Trinidad (2002–2004). *Annals of Tropical Medicine & Parasitology*, 101, pp.69-77.
16. Correa, P.R., Franca, E. & Bogutchi, T.F. 2005. Aedes aegypti infestation and occurrence of dengue in the city of Belo Horizonte, Brazil. *Revista de Saude Publica,* 39, pp.33-40.
17. Fernández, R. W. et al., 2005. Population behavior of *Aedes aegypti* larvae for estimating dengue fever cases in Yurimaguas, Peru, 2000- 2004. *Revista Peruana Medicina Experimental y Salud Publica*, 22, pp.175-182.
18. Arboleda, S.S., Jaramillo, N.N. & Peterson, A.T.A., 2012. Spatial and temporal dynamics of *Aedes aegypti* larval sites in Bello, Colombia. *Journal of Vector Ecology*, 37, pp.37-48.

CHAPTER 3

SYSTEMATIC REVIEW OF VECTOR CONTROL TOOLS FOR DENGUE OUTBREAK PREVENTION AND CONTROL: LACK OF EFFECTIVENESS OF LACK OF EVIDENCE?

ABSTRACT

Background

Vector control tools are thought to impact dengue vectors sufficiently to reduce dengue transmission, however evidence to support this is lacking. Further research is needed to justify the vast resources spent worldwide on vector control tools for dengue.

Methodology/Principal Findings

979 records were identified following searches; 41 were included in this systematic review, of which 19 were analysed in meta-analyses. Inclusion criteria included any study design that included the outcomes mosquito indices and/ or dengue incidence. Reduced odds of dengue incidence were observed for house screening (Pooled OR: 0.22 (95% CI 0.05, 0.93)). The odds of dengue incidence were also reduced in one study for community-based environmental management (OR 0.22, (95% CI 0.15, 0.32)). Among cluster-randomised controlled trials (CRCT), 3 community-based combination interventions significantly impacted mosquito indices: Breteau Index (BI) Rate Ratio (RR) 0.48 (95% CI 0.26, 0.89); BI, RR 0.65 (95% CI 0.52, 0.81); BI, Mean difference (MD) -4.66 (-5.89, -3.43). Insecticide treated curtains did not significantly reduce the BI: Pooled MD -25.16 (95% CI -76.03, 25.71). Among non-RCTs: community-based larviciding significantly reduced the rate of dengue incidence in intervention groups, RR 0.19 (95% CI 0.12, 0.30). Fogging significantly reduced the circulating mosquito population: MD -13.96 (95% CI -21.96, -5.94). Indoor residual spraying insignificantly reduced pooled odds of dengue incidence by 0.67 (95% CI 0.22, 2.11).

Conclusions/Significance

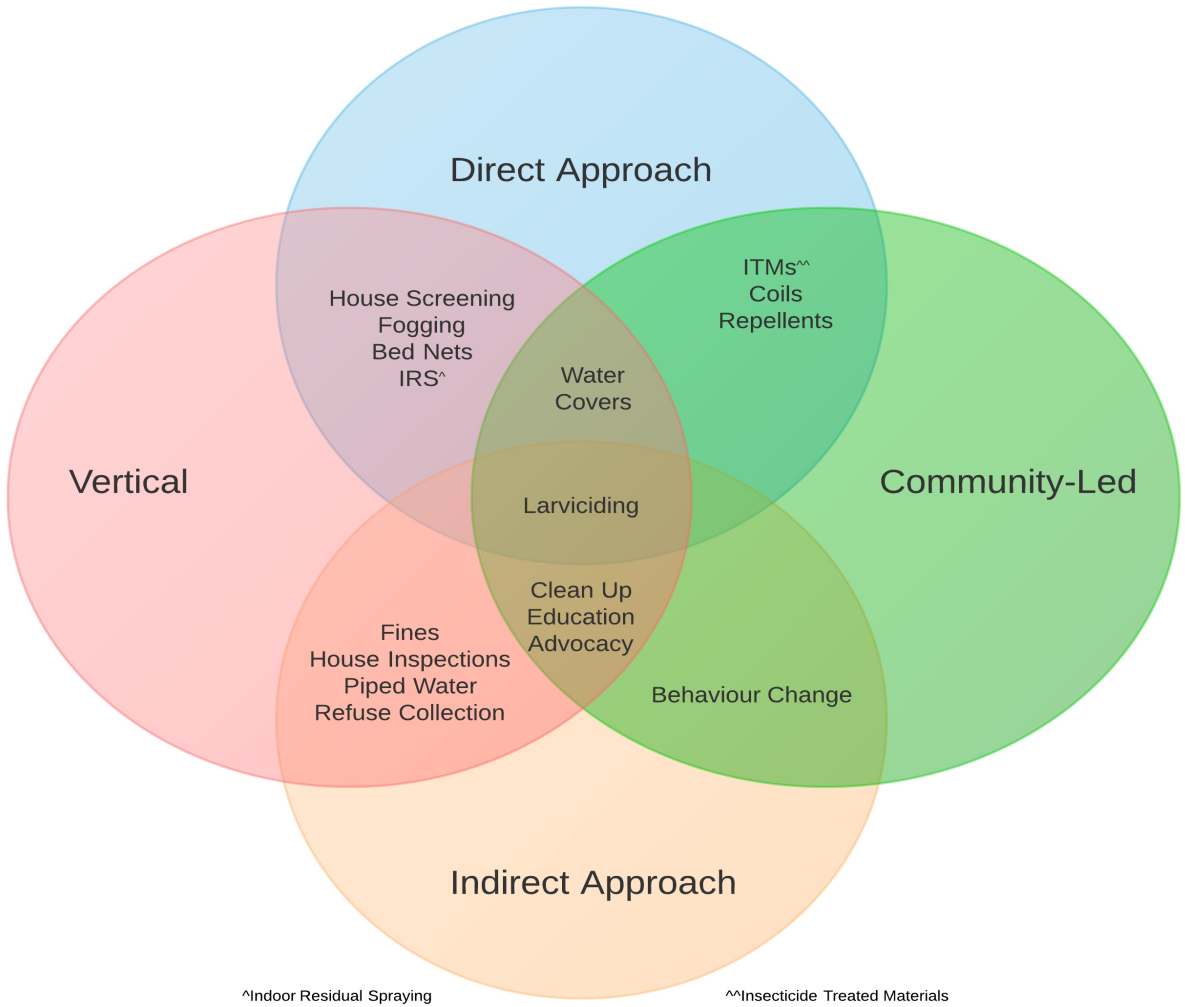
House screening is supported by suggestive evidence that it can reduce the odds of dengue incidence. Community-based combination campaigns can significantly impact vector metrics, with some evidence that they can also impact dengue transmission. Limited evidence exists for the impact of outdoor fogging on circulating mosquito populations, and further research is required to establish whether this translates into an impact on dengue incidence

INTRODUCTION

Dengue is showing signs of emergence in temperate latitudes (Tomasello & Schlagenhauf 2013; Eurosurveillance 2013; Añez & Rios 2013; Messenger et al. 2014) and is a particular threat to many of the international mass-gatherings that are a feature the modern era, such as the FIFA World Cup and the Olympics, or religious gatherings like the Hajj, although the fear of large outbreaks and subsequent global spread from such events has not yet been realised (Shibl et al. 2012; M. E. Wilson et al. 2014). Despite considerable effort and undoubted progress (Da Costa et al. 2014; Osorio et al. 2014; Capeding et al. 2014), a protective vaccine for tourists and endemic communities is not yet available. Consequently, dengue remains unique among the major vector-borne diseases, in that prevention from infection can only be achieved by reducing or eliminating bites by infected vector mosquitoes (World Health Organisation 2009), and that, once infected, no curative treatment exists (Guzman et al. 2010).

Control of dengue vectors can be directed against the immature aquatic stages (larvae and pupae) or the adult mosquitoes, with a number of methods available for each approach. Listed and described elsewhere (World Health Organisation 2009; McCall & Kittayapong 2007; Achee et al. 2015), they are summarised in Figure 3.1 according to whether they target the vector directly (*i.e.* aim to kill or prevent breeding or biting) or indirectly (*e.g.* house or environmental improvements that lead to reduced vector breeding). Also, consideration is given to whether they depend on skilled staff or dedicated resources (equipment, insecticides, transport) (vertical approach) in order to be delivered effectively, or whether affected communities, empowered through education and advocacy, can mobilize and mount effective control operations independently (community-led). Hence, source reduction can be achieved via a horizontal or community-based approach with householders and communities taking responsibility, supported by education and social mobilization. Space-spraying and larviciding on the other hand, require trained personnel to deliver potentially toxic insecticides using specialized equipment and are dependent on vertical municipality-driven vertical programs (World Health Organisation 2009).

Figure 3.1. Venn Diagram describing known dengue interventions and the relationship with policy makers (Vertical/ Community-Led) and how each intervention impacts the mosquito (Direct/ Indirect)



As discussed in Chapter 1, in dengue-affected communities worldwide, immature vector populations are targeted through the elimination of potential breeding sites, typically by collection of purposeless or discarded containers in ‘clean-up’ or environmental management campaigns, while functional sites are either covered (water storage containers), drained (gutters or channels) or treated with an appropriate insecticide (usually referred to as ‘larviciding’) or biological control agent (predatory copepods or fish). Identification of and targeted action towards ‘productive’ container types *i.e.* those which are assessed as contributing the greatest burden of pupae, relative to other containers in the area, can potentially enable more cost-effective larval control (Nathan et al. 2006; Manrique-Saide et al. 2011).

The typical response to dengue outbreaks is to target the adult mosquito population by space-spraying or fogging with insecticide, delivered either outside or inside the home, with the aim of drastically reducing the vector population at the time of delivery. This method is not designed to deliver effective levels of insecticide residue on treated surfaces, and must be repeated at intervals that coincide with the vector life cycle, if the outbreak continues (World Health Organisation 2009).

A number of reviews have examined the evidence for the effectiveness of some methods (Erlanger et al. 2008; Pilger et al. 2010; Esu et al. 2010; Horstick et al. 2010). Erlanger *et al.* (2008) (Erlanger et al. 2008) reviewed data on the effectiveness on vector indices of all vector control methods and concluded that integrated vector control was the most effective, while environmental management had minimal impact. Notably, the evidence for impact of outdoor space-spraying was limited, though only 1 of the studies included was less than 30 years old (dated from 2015). Two subsequent reviews (Esu et al. 2010; Pilger et al. 2010) focused on peri-domestic space spraying and concluded that there was no evidence to support its use in dengue outbreak control, either as a standalone intervention or in combination with other interventions. Horstick *et al.* (2010) (Horstick et al. 2010) also found no evidence for a demonstrable effect of vector control on entomological indices and concurrently identified specific weaknesses in funding, management, staffing and community engagement, all of which conspired to lower operational standards and ultimately restrict any likelihood of success.

Today, dengue outbreaks occur frequently worldwide, and many local authorities continue to implement existing vector control strategies, whether or not they have confidence in the potential for success. A particular need in at risk communities are tools that can be deployed to respond rapidly to the growing number and intensity of dengue outbreaks. What are the best dengue vector control tools? Are previous dengue control failures the result of low operational and management strategies, or are the available tools simply not effective? A systematic review was undertaken to answer these questions and others, and to provide guidance on the most effective strategies currently available to combat dengue.

METHODS

Objectives

To systematically review randomized and non-randomized studies to evaluate the evidence of the effectiveness of vector control interventions in a) reducing vector indices and b) preventing dengue transmission.

Eligibility Criteria

Table 3.1 displays the eligibility criteria. Studies that presented data for a minimum duration of 3 months were included, as this was deemed necessary to be able to demonstrate sustained impact on the vector population and, if measured, a subsequent impact on dengue transmission. In addition, only studies published since after 1980 were considered eligible for inclusion, for a number of reasons. The period after 1980 saw the expansion in urban populations worldwide, notably in the less developed countries where migration from urban to rural areas dramatically altered the urban to rural population ratio (Alirol et al. 2011; Gubler 2002). This also was the beginning of the 'globalization' era, as characterized by steep increases in trans-national and international movement of humans and merchandise, and the time when all four dengue serotypes were reported in every continent, leading to an increase in the frequency and magnitude of dengue outbreaks (Simmons et al. 2012; Gubler 2011;

San Martin et al. 2010). The authors are cognisant of the achievements prior to 1970, such as the ambitious yellow fever program when *Aedes aegypti* populations were significantly diminished or eliminated across most regions of Latin America, primarily by peri-focal spraying with DDT and source reduction (World Health Organisation 2012ab; World Health Organisation 2012aa; Simmons et al. 2012; Soper 1967). On balance, it was concluded that the control tools available before the 1980s and the environments in which they were carried out, were not pertinent to the challenge of dengue control in urban environments of the 21st century, based on the significant logistical, sociological and epidemiological changes, concomitant with the rise of insecticide resistance in vector populations (Ranson et al. 2010; Luz et al. 2011).

Table 3.1. Criteria for inclusion or exclusion of studies.

	Inclusion Criteria	Exclusion Criteria
Study design	Any randomised or non-randomised study design.	Review articles or opinion papers
	Primary research and models using empirical data.	Non-empirical research/ modelled data
Mosquitoes	*Aedes aegypti/ albopictus*	All other mosquito spp.
Interventions	Any study where vector control tools (singly or combined) were used for >3 months for the duration of the outbreak	
Outcomes	Any study with empirical data reporting dengue case numbers and/or entomological indices monitored longitudinally for the duration of the intervention	Entomological data without longitudinal (interval) data capture
	Dengue cases reported either by the study or obtained from external institutions (e.g. hospital records)	Qualitative dengue reports
Other	Papers published from 1980 onwards	Papers published pre-1980

Outcomes

The primary outcome was dengue incidence; secondary outcomes were Breteau Index (BI), House Index (HI), Container Index (CI), tank positivity, number of mosquito adults, pupae per person index (PPPI), presence of *Aedes* immatures and ovitrap positivity rates.

All methods were pre-specified in the review protocol. PRISMA guidelines Group were followed (Moher et al. 2009; Higgins & Green 2015).

Search Strategy

The original search was conducted in April 2012 and then updated December 2013 and again in January 2015. The databases WHOLIS, MEDLINE, EMBASE, LILACS and Science Citation Index were searched using the Medical Subject Heading (MeSH) "dengue" followed by the Boolean operator "and" combined with the following 'free text' terms "epidemic" and further combined in succession with: 'threshold' 'sentinel' 'early warning' 'case management' 'vector control' 'DDSS' 'space spraying' 'indoor residual spraying' 'fogging' 'integrated vector management' 'IVM' 'source reduction' 'container' 'larvicide' 'repellent' 'insecticide' 'adulticide' 'fumigant' 'aerial spraying' 'dengue decision support system'. The reference list of each of the included studies was also searched, and ''grey literature'' (cited unpublished documents) were sought by communication with authors. No limits were placed on year of publication status or language.

Study Selection

Search results were imported into EndNote (EndNote X5, Build 7473). LRB and PJM independently assessed the title and abstract of each record (or the corresponding full article) retrieved by the search for eligibility; any discrepancies were discussed. The full article was retrieved for each eligible study. The study's investigators were contacted if eligibility was unclear, additional data were unpublished or the article was inaccessible. Each article was scrutinized to detect multiple publications from the same trial; such publications were included as a single study.

Data Extraction

LRB and PJM independently extracted data according to an agreed checklist (Appendix 3) and differences were discussed. Trial characteristics and risk of bias information were extracted along with outcome data. For each randomized controlled trial, we extracted the number of human individuals randomized and the number of human individuals analysed for each treatment group. For dichotomous outcomes, we extracted the number of individuals experiencing the event in each treatment group for each study. For continuous outcomes, such as mosquito indices, we extracted means and standard deviations (where presented) or medians, interquartile ranges, and ranges. When such data were not reported we extracted narrative information and tabulated results. For non-randomized studies, we extracted measures of effect, as well as treatment group data.

Risk of Bias Assessment

Using a pre-piloted form, LRB and PJM independently assessed risk of bias and discussed differences (Appendix 4). Those studies that were not included in meta-analyses were still analysed for risk of bias (Appendix 4); additional descriptive results are presented in Appendix 5.

For randomized controlled trials we used the Cochrane risk of bias tool and addressed: random sequence generation; allocation concealment; blinding; incomplete outcome data, selective outcome reporting, and other biases (Higgins & Green 2015) (Appendix 6). For each component, for each trial, a judgment of high, low, or unclear risk of bias was made and the rationale for the judgment was given (Appendix 6, Appendix 7). For non-randomized studies, LRB and PJM used the Quality Assessment Tool for Quantitative Studies (Thomas 2004) (Appendix 4). This ensured each study could be ranked according to inherent study design limitations, which included but were not limited to, bias, confounding and blinding.

Data Analyses

Analyses were performed in Review Manager (RevMan Version 5.2. Copenhagen: The Nordic Cochrane Centre, 2012). We extracted the measure of effect and CI from the study reports. Where possible, we stratified analyses by intervention, outcome, measure of effect and study design. For multi-arm trials, data from numerous intervention groups were pooled. For multi-arm trials, data from numerous intervention groups were pooled. We calculated trial-level results (*i.e.* MD, RR or OR and standard error [SE]) and pooled them using random-effects inverse-variance meta-analysis to account for large variability present between studies. Results are presented in forest plots. Sub-group analyses were used to stratify studies that used different and/ or combination interventions.

Heterogeneity was assessed using the I^2 test statistic, the chi-squared test ($P<0.10$ indicated possible significance due to the low power of the test) and by visual inspection of the forest plots to identify overlapping confidence intervals.

When heterogeneity was detected, possible causes were explored using subgroup analyses and predefined covariates.

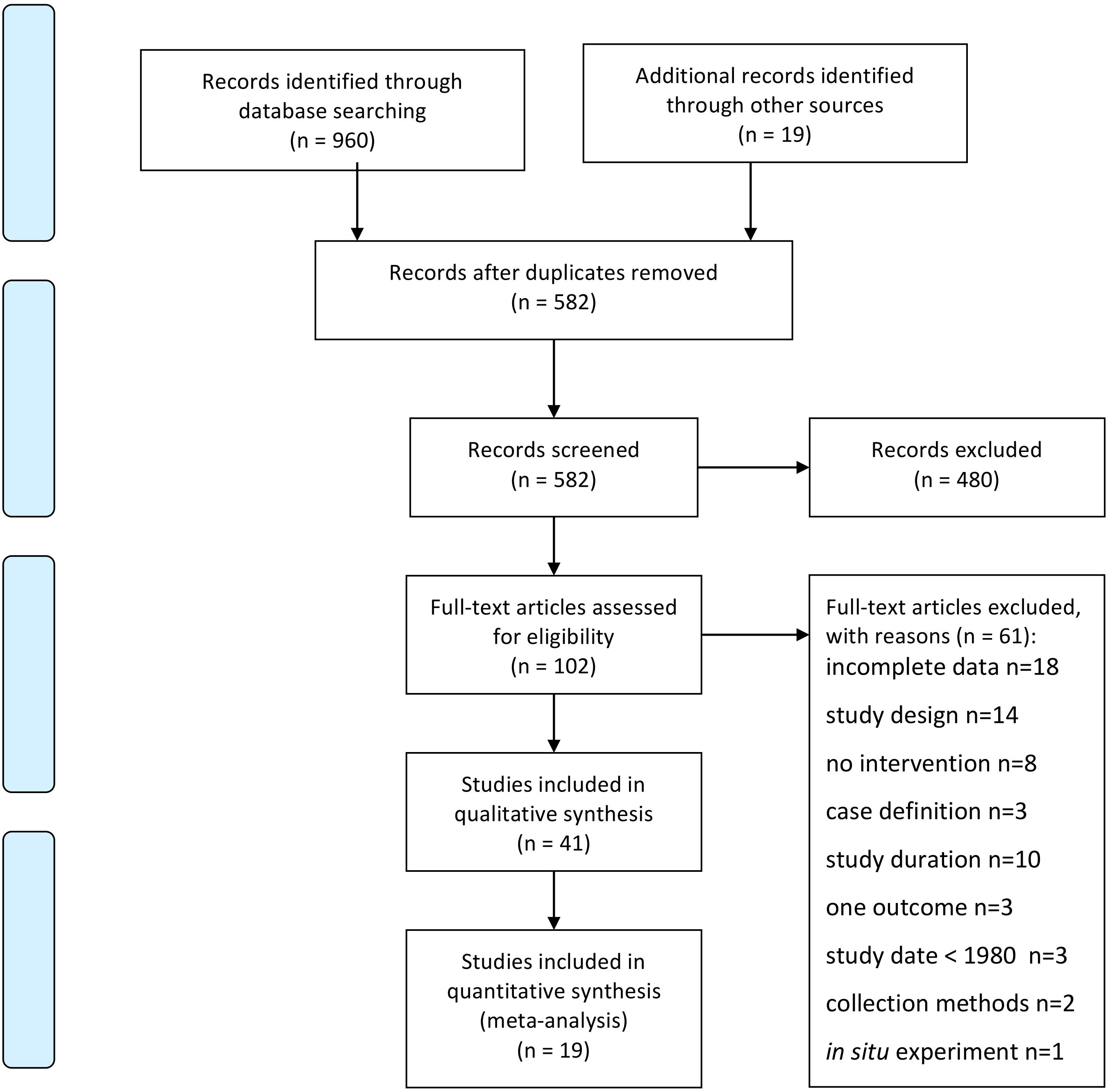
Subgroup analyses were planned to explore potential sources of heterogeneity: (*i.e.* effect of seasonality, mosquito spp., duration of intervention, coverage); analyses were not carried out because of the low number of studies in analyses. Similarly, pre-planned sensitivity analyses excluding studies with a high risk of bias to assess the robustness of results were not carried out and the pre-planned funnel plots were not constructed to explore publication biases.

RESULTS

Study Eligibility Results

Figure 3.2 displays the flow diagram. A total of 960 records were identified by the search, plus 19 from other sources (Figure 3.2). After removing duplicates, 582 citations were screened, of which 480 were excluded. The full texts of the remaining 102 records were assessed and 61 articles were excluded. The reasons for exclusion were: incomplete data on intervention or dengue cases (18 studies); study was a review, non-peer reviewed report or mathematical model (14 studies); no intervention was carried out (eight studies); undefined or inadequate dengue case definition (three studies); intervention or outbreak duration was less than 3 months (10 studies); study included only one required outcome (three studies); study preceded 1980 (three studies); time series data collection not reported (two studies); *in situ* experiment (one study). Forty-one studies were included in the review (Appendix 5) (1-41).

Figure 3.2. PRISMA 2009 flow diagram. Diagram of searches performed and the number of articles returned and examined at each stage.



Characteristics of Included Studies

Appendix 5 displays the main characteristics of included studies. Of the 41 included studies, geographic study locations comprised: SE Asia (n=11) or Central America (n=10), South Asia (n=8), Australasia (n=4), South America (n=5) and North America (n=3). All studies were published between 1986 and 2014, and 2009 was the median year of publication.

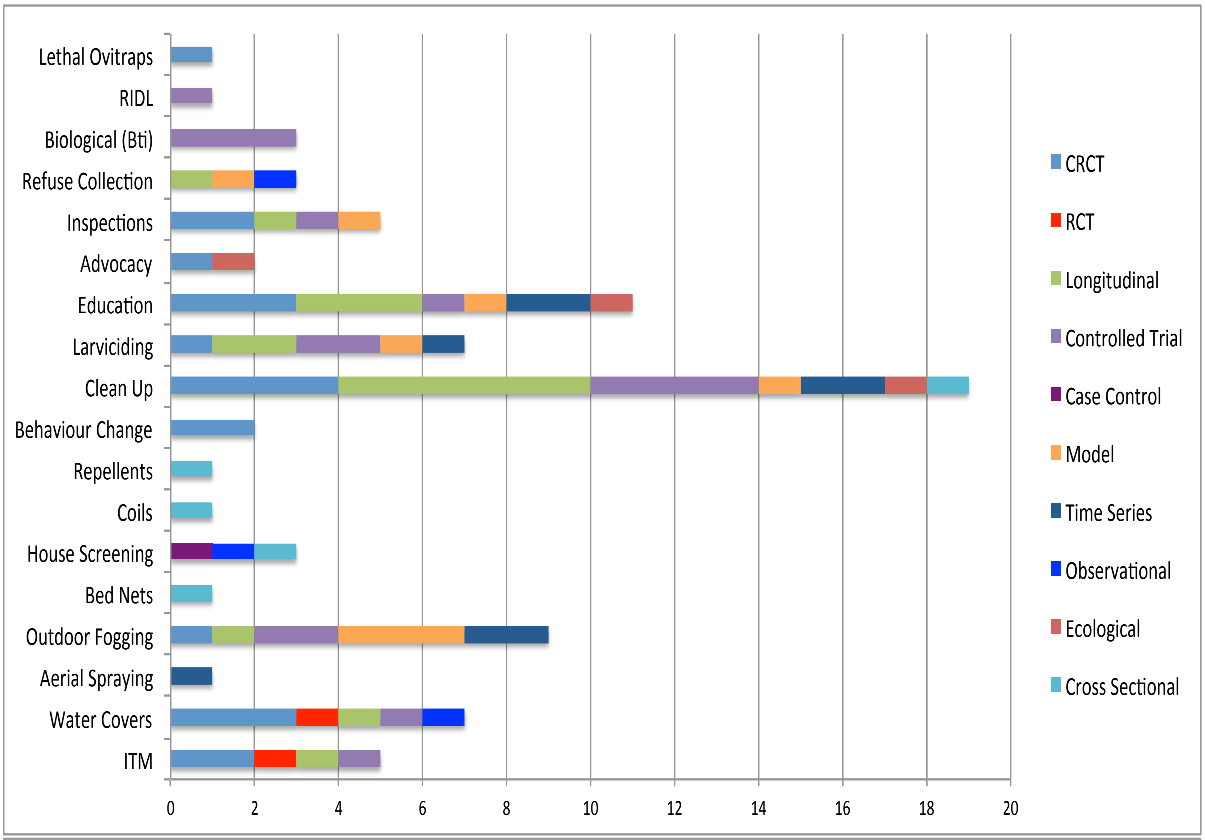
Grouped by study design, the studies comprised: 9 randomised controlled trials (*i.e.* 7 cluster-randomized and 2 randomized controlled trials) and 32 non-randomised studies (*i.e.* 8 controlled trials, 7 longitudinal studies, 4 interrupted time series studies, 5 before and after studies, 2 observational studies, 1 case-control study, 1 cross sectional study, 1 retrospective observational study, 1 ecological study and 2 models) (Appendix 5).

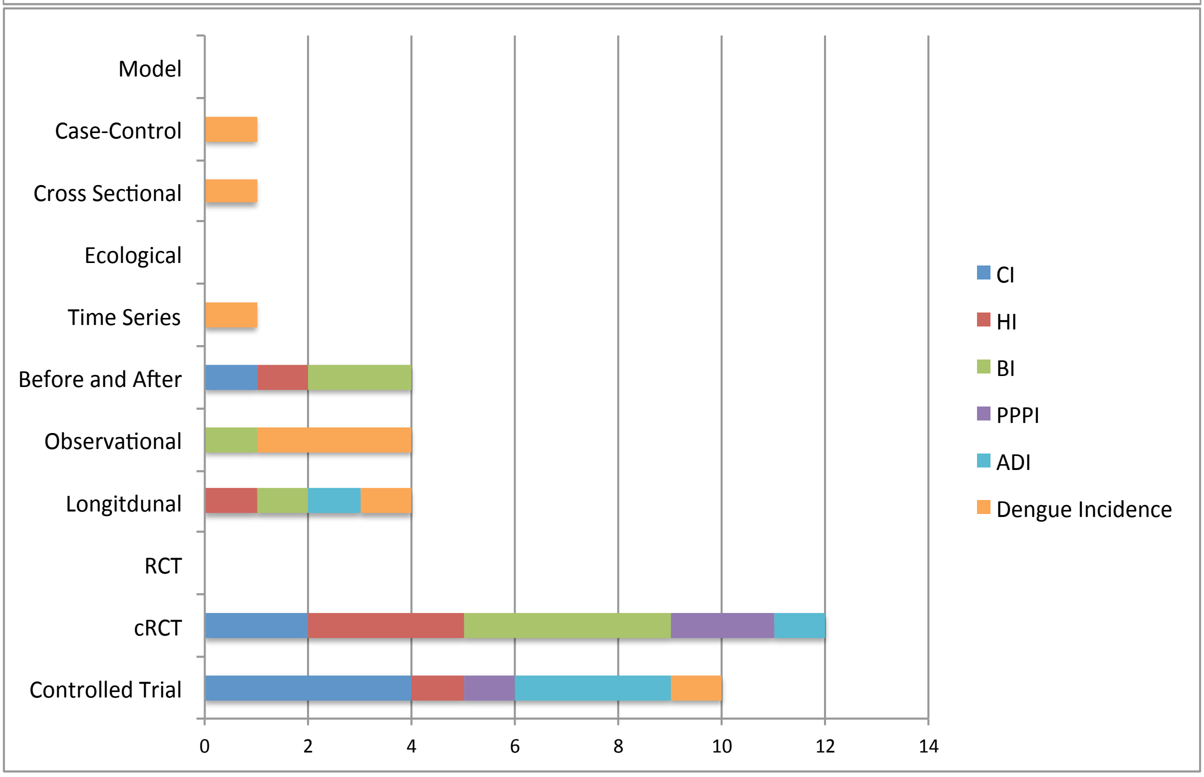
All studies presented data on *Aedes aegypti*, while four of these also presented data on *Aedes albopictus*.

Vertical and community-led interventions were used exclusively in 20 and 10 studies respectively, while 11 studies used a combination of both (Appendix 5). Multiple interventions (23 studies) were more common than single interventions (18 studies) (Appendix 5). Study duration ranged from 5 months to 10 years, of which 16 studies were less than 1 year, 12 took place over 1-3 years and 7 were 8 or more years in duration.

Figure 3.3 (top) summarises the frequency of vector control tools by study design. It is clear from the graph that the most frequently evaluated intervention was clean up campaigns (n=19), of which 4 were included in CRCTs. Outdoor fogging (n=9), education (n=11), larviciding (n=7) and water covers (n=7) were also popular interventions. Note that the frequency of trial design is not accurately represented, as each study design can evaluate >1 intervention. Figure 3.3 (bottom) also summarises the reported reduction in outcome at a statistically significant level ($p<0.05$). Notably, the frequency of success in reducing dengue incidence following vector control interventions is lower in more robust study designs; of the randomised study designs that explored dengue incidence as an outcome (n=2), 0 reported a statistically significant reduction, compared with 14 remaining study designs, of which 8 reported a statistically significant reduction.

Figure 3.3. Top: Histogram of frequency of interventions used throughout the 41 studies, by study design. Bottom: Histogram of frequency of study design that reported a significant (p<0.05) reduction in the outcome. PPPI = Pupae Per Person Index. ADI = Adult Mosquito Density Index.





18 studies reported dengue incidence, 17 studies reported BI, 16 studies reported HI, 11 studies reported CI, 1 study reported tank positivity, 3 studies reported number of mosquito adults, 6 studies reported pupal indices, and 3 studies reported ovitrap data.

Risk of bias assessment results

Non-randomised studies

Appendix 4 displays the results of this assessment for non-randomised studies. Nineteen studies scored a 3, which is equal to a weak study, while nine studies scored a 2, equal to a moderate study, and only two studies scored a 1, equal to a strong study.

Randomised studies

Appendix 6 and Appendix 7 display the results of this assessment. Nine studies were at low risk of bias for selective outcome reporting and the remaining study was at unclear risk. Seven studies were at low risk of bias for incomplete outcome data, while one was at medium risk and one was at a high risk of bias. There was a high risk of bias via blinding in all studies. Risk of bias through allocation concealment was low in one study, unclear in four studies and high in four studies. Risk of bias attributed to generation of allocation sequence was low in four studies, unclear in four studies and high in one study.

Effectiveness of interventions

Nineteen studies (2-4,8,10,11,14,15,22,25,29,30,32,33,36-39,41) provided sufficient data to allow their inclusion in meta-analyses. The results of those analyses are presented here stratified by reported outcome, either the impact on dengue incidence or on vector indices.

Impact on Dengue Incidence

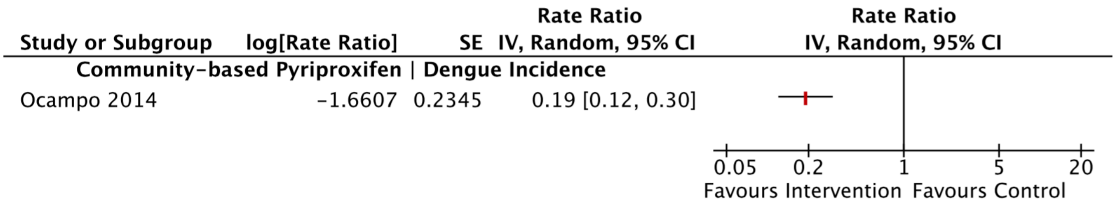
Impact of Dengue Incidence in Randomised Controlled Trials

No randomised controlled studies exploring the impact of vector control on dengue incidence were present.

Impact on Dengue incidence in Non-RCTs

Pyriproxifen as part of a community-based strategy significantly reduced the rate of dengue incidence in the intervention group: RR 0.19 (95% CI 0.12, 0.30) (Figure 3.4) (36).

Figure 3.4. Forest Plot of Comparison: Quasi-experimental study on community participation using pyriproxifen vs. control, outcome: dengue incidence.

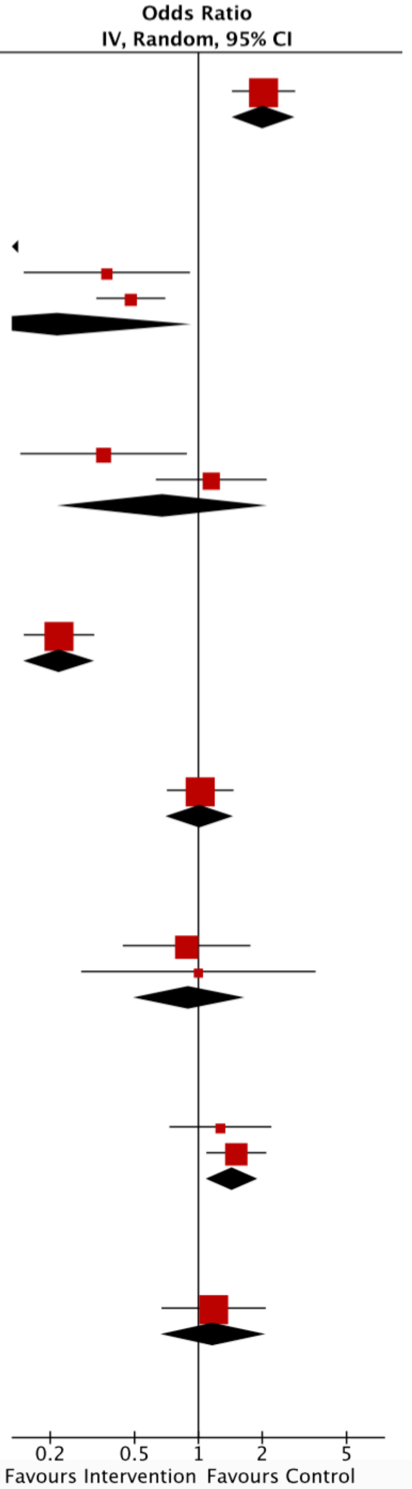


Five studies measuring the impact of any intervention on dengue incidence using odds ratios were included in one meta-analysis (Figure 3.5). These included a number of study designs (cross sectional, 2 x observational, retrospective observational, case-control) and interventions (knockdown sprays *i.e.* insecticidal aerosols, house screening, indoor residual spraying, community-based environmental management, insect repellents, bed nets, mosquito coils and mosquito traps). Across the studies, heterogeneity was marked, likely due to the varying study designs, number of studies per subgroup and intervention type (I^2 = 92.1%).

The presence of house screening in homes (three studies (8,15,25)) significantly reduced the odds of dengue incidence compared to homes without screens (0.22: 95% confidence interval (CI) 0.05, 0.93; p=0.04). Combined community-based environmental management together with the use of water container covers (14) also reduced the odds of dengue incidence to 0.22 (95% CI 0.15, 0.32; p<0.0001).

Figure 3.5 Forest Plot of Comparison: non-RCT sub-group analysis stratified by intervention vs. control, outcome: Dengue incidence

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
Knockdown Sprays				
McBride, 1998	0.708	0.1752	100.0%	2.03 [1.44, 2.86]
Subtotal (95% CI)			**100.0%**	**2.03 [1.44, 2.86]**
Heterogeneity: Not applicable				
Test for overall effect: Z = 4.04 (P < 0.0001)				
House Screening				
Murray-Smith 1996	-2.8061	0.2708	34.0%	0.06 [0.04, 0.10]
Ko, 1992	-0.9943	0.4607	31.3%	0.37 [0.15, 0.91]
McBride, 1998	-0.734	0.1912	34.8%	0.48 [0.33, 0.70]
Subtotal (95% CI)			**100.0%**	**0.22 [0.05, 0.93]**
Heterogeneity: $Tau^2 = 1.53$; $Chi^2 = 39.85$, df = 2 (P < 0.00001); $I^2 = 95\%$				
Test for overall effect: Z = 2.06 (P = 0.04)				
Indoor Residual Spraying				
V.-Prokopec 2010	-1.0297	0.4616	45.7%	0.36 [0.14, 0.88]
Ko, 1992	0.1398	0.307	54.3%	1.15 [0.63, 2.10]
Subtotal (95% CI)			**100.0%**	**0.67 [0.22, 2.11]**
Heterogeneity: $Tau^2 = 0.53$; $Chi^2 = 4.45$, df = 1 (P = 0.03); $I^2 = 78\%$				
Test for overall effect: Z = 0.68 (P = 0.50)				
Environmental Management | Water Lids (community-based)				
Toledo, 2011	-1.5141	0.1954	100.0%	0.22 [0.15, 0.32]
Subtotal (95% CI)			**100.0%**	**0.22 [0.15, 0.32]**
Heterogeneity: Not applicable				
Test for overall effect: Z = 7.75 (P < 0.00001)				
Insect Repellents				
McBride, 1998	0.0198	0.1848	100.0%	1.02 [0.71, 1.47]
Subtotal (95% CI)			**100.0%**	**1.02 [0.71, 1.47]**
Heterogeneity: Not applicable				
Test for overall effect: Z = 0.11 (P = 0.91)				
Bed Nets				
McBride, 1998	-0.1278	0.3537	77.1%	0.88 [0.44, 1.76]
Ko, 1992	0	0.6495	22.9%	1.00 [0.28, 3.57]
Subtotal (95% CI)			**100.0%**	**0.91 [0.49, 1.67]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.03$, df = 1 (P = 0.86); $I^2 = 0\%$				
Test for overall effect: Z = 0.32 (P = 0.75)				
Mosquito Coils				
Ko, 1992	0.239	0.2825	25.7%	1.27 [0.73, 2.21]
McBride, 1998	0.4121	0.1663	74.3%	1.51 [1.09, 2.09]
Subtotal (95% CI)			**100.0%**	**1.44 [1.09, 1.91]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.28$, df = 1 (P = 0.60); $I^2 = 0\%$				
Test for overall effect: Z = 2.56 (P = 0.01)				
Mosquito Traps				
Ko, 1992	0.1655	0.2888	100.0%	1.18 [0.67, 2.08]
Subtotal (95% CI)			**100.0%**	**1.18 [0.67, 2.08]**
Heterogeneity: Not applicable				
Test for overall effect: Z = 0.57 (P = 0.57)				



Test for subgroup differences: $Chi^2 = 88.09$, df = 7 (P < 0.00001), $I^2 = 92.1\%$

In relation to Figure 3.5, Toledo 2011: original risk ratio was assumed to be similar to the odds ratio, which may bias in favour of the intervention. McBride 1998: (a cross-sectional study design (no control group)) insect repellents, upper confidence limit was corrected from 1.44 to 1.47 by RevMan. Ko 1992: mosquito traps, upper confidence limit was altered by Revman from 2.05 to 2.08; mosquito coils, upper confidence limit altered by RevMan from 2.22 to 2.21; house screens, confidence limit altered by RevMan from 0.89 to 0.91. Vasquez-Prokopec et al., 2010, IRS odds ratios relates to secondary dengue infections only.

Indoor residual spraying reduced the odds of infection to 0.67 (95% CI 0.22, 2.11), but the result was not significant (p = 0.50) (22,25). There was no evidence that the use of mosquito repellents (8), bed nets (8,25) or mosquito traps (25) significantly increased or reduced the odds of dengue infection, with odds ratios of 1.02 (95% CI 0.71, 1.47; p=0.91), 0.91 (95% CI 0.49, 1.67; p=0.75) and 1.18 (95% CI 0.67, 2.08; p=0.57) respectively.

Conversely, the use of knockdown sprays (8) (OR 2.03 (95% CI 1.44, 2.86)) or mosquito coils (8,25) (OR 1.44 (95% CI 1.09, 1.91; p = 0.01)) was significantly associated with an increased odds of dengue incidence.

Impact on vector indices

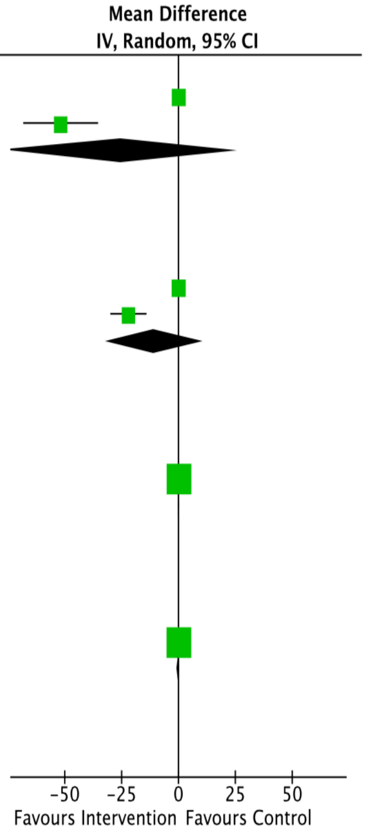
Impact on mosquito indices evaluated in cluster-randomized controlled trials (CRCTs)

Cluster-randomized controlled trials investigating the efficacy of insecticide-treated curtains (ITCs) (32,33) and community-based combination interventions (combined uses of: waste disposal, clean up campaigns, formation of community working groups, mobilization of school children and education (29); source reduction, larviciding, entomological surveillance, adulticiding, communication, education and punitive fines (3) were included in these meta-analyses. Forest plots of analyses measuring impact on the BI, HI, CI and pupal indices and is shown in Figures 3.5-3.7.

In Figure 3.6, the use of insecticide treated curtains (32,33) did not significantly reduce the pooled mean difference for either the Breteau Index, -25.16 (95% CI -76.02, -25.70; p=0.33), House Index, -10.58 (95% CI -32.22, -11.05; p=0.34), Container Index -0.24 (95% CI -0.16, 0.25) or pupae per person index at -0.19 (95% CI -0.37, 0.75). Heterogeneity between the studies was high, with I_2 = 97% (p<0.0001) for outcomes BI and HI.

Figure 3.6. Forest Plot of Comparison: CRCTs sub-group analysis for insecticide-treated curtains intervention vs. control, outcomes: Breteau Index, House Index, Container Index, Pupae Per Person Index.

	Intervention			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Insecticide Treated Curtains | BI								
Lenhart 2013	4.2762	0.7616	13	4.1502	0.3676	13	51.3%	0.13 [−0.33, 0.59]
Vanlerberghe 2013	25.8	23.3464	22	77.6	54.9158	66	48.7%	−51.80 [−68.25, −35.35]
Subtotal (95% CI)			**35**			**79**	**100.0%**	**−25.16 [−76.03, 25.71]**
Heterogeneity: Tau² = 1312.89; Chi² = 38.23, df = 1 (P < 0.00001); I² = 97%								
Test for overall effect: Z = 0.97 (P = 0.33)								
Insecticide Treated Curtains | HI								
Lenhart 2013	3.7615	0.499	13	3.675	0.3327	13	51.7%	0.09 [−0.24, 0.41]
Vanlerberghe 2013	17.5	13.9836	22	39.5	22.3731	66	48.3%	−22.00 [−29.95, −14.05]
Subtotal (95% CI)			**35**			**79**	**100.0%**	**−10.58 [−32.22, 11.05]**
Heterogeneity: Tau² = 235.66; Chi² = 29.56, df = 1 (P < 0.00001); I² = 97%								
Test for overall effect: Z = 0.96 (P = 0.34)								
Insecticide Treated Curtains | CI								
Lenhart 2013	2.6381	0.651	13	2.3984	0.3824	13	100.0%	0.24 [−0.17, 0.65]
Subtotal (95% CI)			**13**			**13**	**100.0%**	**0.24 [−0.17, 0.65]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.14 (P = 0.25)								
Insecticide Treated Curtains | PPPI								
Lenhart 2013	2.3249	0.8065	13	2.1357	0.6451	13	100.0%	0.19 [−0.37, 0.75]
Subtotal (95% CI)			**13**			**13**	**100.0%**	**0.19 [−0.37, 0.75]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.66 (P = 0.51)								



Community-based combination interventions significantly impacted the BI and HI: rate ratio 0.48 (95% CI 0.26, 0.89) and 0.49 (95% CI 0.27, 0.89) (Figure 3.7) (3), while in Castro *et al.* (2012), routine interventions led by the community were significantly more effective than routine interventions alone (RR 0.65 (95% CI 0.52, 0.81)) (39) (Figure 3.7). Similarly, in Arunachalam *et al.* (2012), the mean difference was also significantly reduced for all metrics: BI -4.66 (-5.89, -3.43), HI -17.10 (-22.16, -12.04) and CI -12.30 (-15.31, -9.29) (Figure 3.8) (29).

Figure 3.7. Forest Plot of Comparison: CRCT community-based environmental management intervention vs. control, outcomes Breteau Index, House Index. CRCT Community empowerment with routine control vs. control (routine control alone), outcome: Breteau Index.

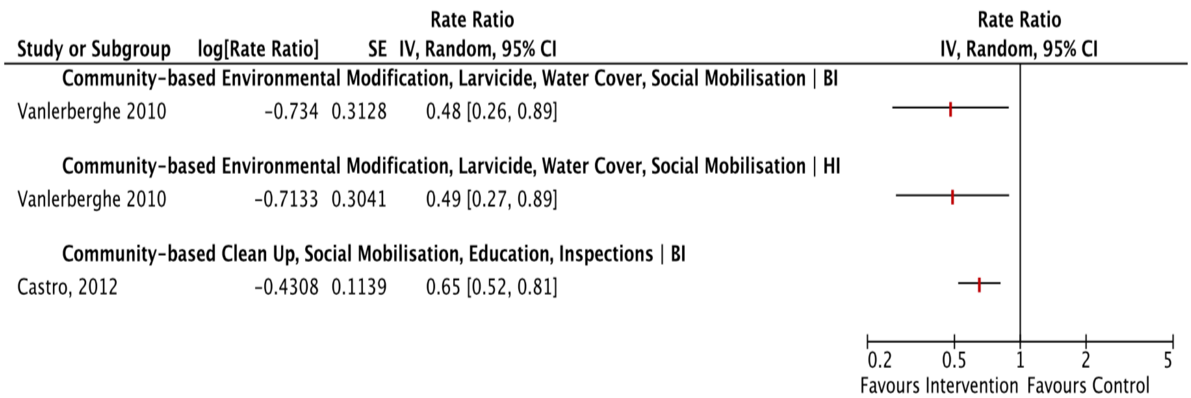
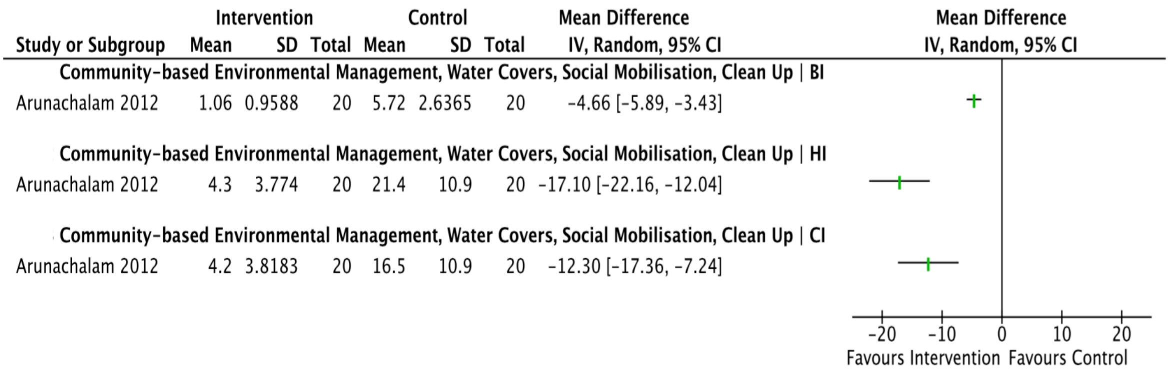


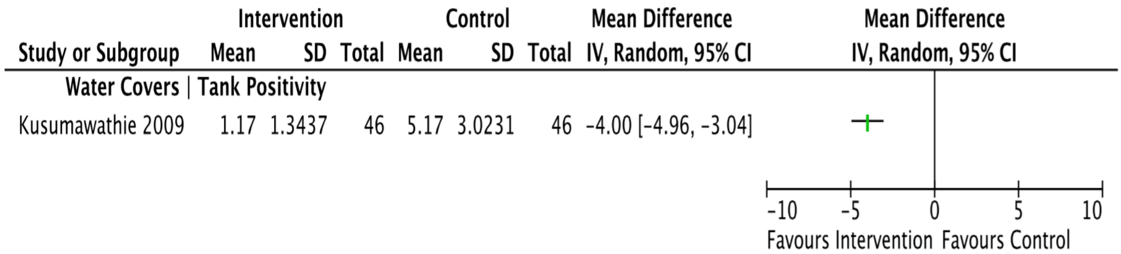
Figure 3.8. Forest Plot of Comparison: CRCT community-based environmental management intervention vs. control, outcomes: Breteau Index, House Index, Container Index.



Impact on mosquito indices evaluated in randomized controlled trials (RCTs)

One study investigated the impact of covering productive breeding container types. Water tank covers significantly reduced the number of tanks positive for immature stage *Ae. aegypti*: MD = -4.00 (95% CI -4.96, -3.04) (10), but an impact on dengue incidence was not evaluated (Figure 3.9).

Figure 3.9. Forest Plot of Comparison: RCT Net covers on water storage tanks vs.. control, outcome: tank positivity.



Impact on mosquito indices evaluated in non-RCTs

These studies evaluated the impact of a number of interventions that were not possible to combine into one forest plot, due to heterogeneity in study design, outcome or outcome measure. The interventions used were: 1) pyriproxifen (36); 2) release of genetically modified (RIDL) mosquitos (41) 3) fogging (30); 4) community-based environmental management (including amongst others, household control of larval habitats, transforming garbage belts, repairing broken water pipes and manufacturing water container covers) (4); 5) clean-up campaigns in conjunction with IRS and larviciding (2); 6) fogging, source reduction and larviciding (11); 7) lethal ovitraps (37); 8) combined use of ITMs as water covers and pyriproxifen (38).

The odds of ovitrap positivity were reduced in the intervention group: OR 0.11 (95% CI 0.07, 0.18) by releasing sterile male mosquitoes into the intervention clusters (41) (Figure 3.10).

Outdoor fogging (nocturnal ultra-low volume fogging using DUET (sumithrin: 5%, prallethin: 1%) significantly reduced the mean number of adult *Ae. albopictus* in the intervention group by -13.90 (95% CI -21.86, -5.94) (Figure 3.11) but did not measure effects on immature stages (30). Sampling was conducted using BioGents Sentinel Trap and fogging was conducted between 3-5 times per year; 43-90% mosquito control was achieved.

Community-based environmental management significantly reduced the House Index: MD = -2.14 (95% CI -3.72, -0.56) (4) (Figure 3.11) and combination interventions (clean-up campaigns in conjunction with IRS and larviciding) reduced ovitrap positivity: MD = -10.30 (95% CI -12.80, -7.80) (2) (Figure 3.11).

The use of fogging, source reduction and larviciding resulted in lower odds of detecting increased larval densities when compared to baseline: Breteau Index OR = 0.15 (95% CI 0.10, 0.24) and House Index OR = 0.13 (95% CI 0.08, 0.22) (11), while the odds of the presence of immature stage *Aedes* were reduced in the intervention group, through the combined use of Olyset net covers for water jars and pyriproxifen for a period of 5 months (Figure 3.12) (38).

Biogents Sentinel lethal ovitraps demonstrated potential in reducing the number of circulating adult mosquitoes, although this result was modest and insignificant: MD 0.30 (95% CI -0.74, 0.13) (Figure 3.13) (37).

Figure 3.10. Forest Plot of Comparison: Non-randomised controlled trial on RIDL mosquitoes vs. control, outcome: ovitrap positivity

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Odds Ratio IV, Random, 95% CI
RIDL | Ovitrap Positivity					
Harris 2012	28	282	131	267	0.11 [0.07, 0.18]

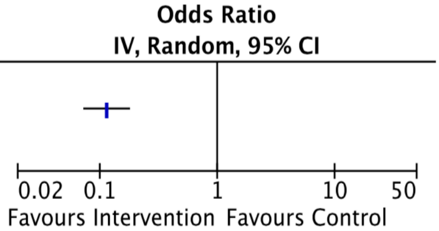


Figure 3.11. Forest Plot of Comparison: Non-RCTs subgroup analysis for multiple interventions vs. control, outcomes: BGS Adult Catch, Breteau Index, Ovitrap Positivity.

Study or Subgroup	Intervention Mean	Intervention SD	Intervention Total	Control Mean	Control SD	Control Total	Mean Difference IV, Random, 95% CI
Fogging | BGS Adult Catch							
Farajollahi 2012	2.5	2.5239	13	16.4	14.4222	13	−13.90 [−21.86, −5.94]
Community-based Environmental Modification, Water Covers, Inspections, Social Mobilisation | BI							
Baly, 2009	0.22	0.71	20	2.36	3.54	20	−2.14 [−3.72, −0.56]
Clean Up, Larvicide, IRS | Ovitrap Positivity							
Hanna, 2001	2.2	1.44	30	12.5	6.85	30	−10.30 [−12.80, −7.80]

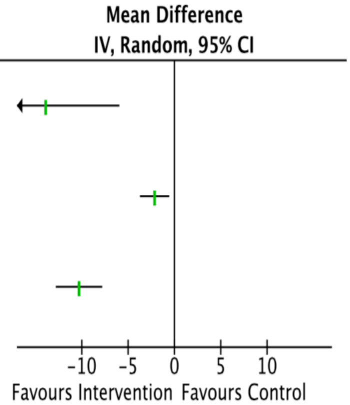


Figure 3.12. Forest Plot of Comparison: Non-RCT multiple interventions vs. baseline, outcome: Breteau Index, House Index. Controlled trial subgroup analysis for larvicide, ULV/ source reduction and Olyset container covers and pyriproxifen vs. control, outcome: HI, BI, Presence of *Aedes* immatures stages.

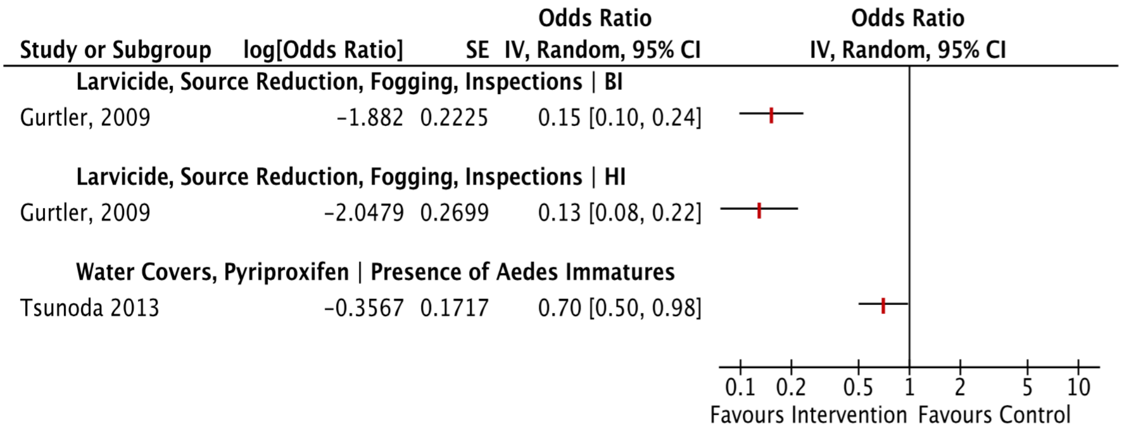
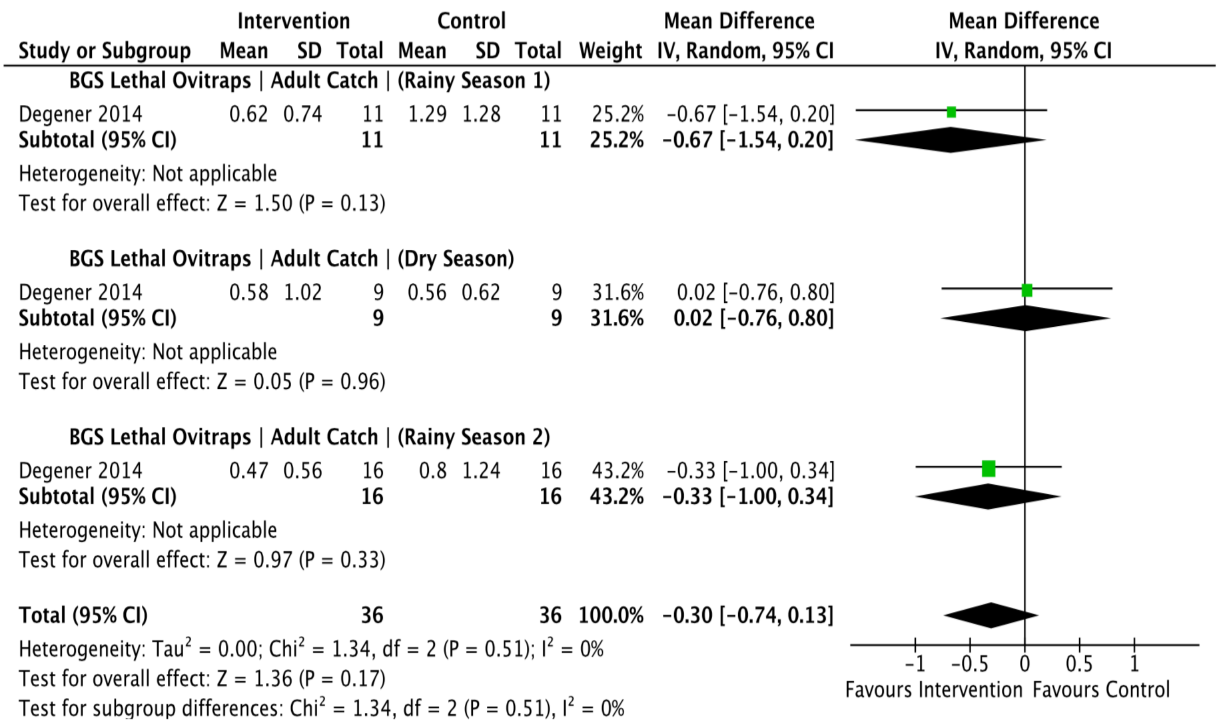


Figure 3.13. Forest Plot of Comparison: CRCT sub-group analysis for BioGents Sentinel Trap vs. control, outcome: number of mosquito adults.



DISCUSSION

This systematic review and meta-analysis covered the past 35 years of research, during which dengue has grown to become of major global public health importance. Perhaps in recognition and response to that increase, 24 of the 41 studies that were eligible for inclusion were published in the past 7 years. However, most of the 41 investigated the impact of different interventions on dengue vector indices alone; 13 studies measured impact on dengue incidence, of which only 6 were suitable for inclusion in meta-analyses.

The strongest evidence for effectiveness in preventing dengue transmission was for house screening. Three studies (8,15,25) were included in a meta-analysis that indicated a significant protective effect (pooled OR: 0.22 (95% CI 0.05, 0.93)) against dengue incidence in screened homes compared to unscreened homes (Figure 3.5). Although the study design of each study somewhat limits the interpretation of this result, still it indicates that house screening can potentially reduce dengue transmission among communities. In light of these results and considering the predominantly endophagic/ endophillic behaviour of *Aedes aegypti* (Perich et al. 2000), it is understandable that limiting mosquito access to houses should be effective in reducing biting by this species. This is apparent in other vector species too - “Mosquito-proofing” houses was first considered over a century ago, while its potential as a sustainable and effective tool for malaria control has been evaluated in randomized controlled trials in recent years (Lindsay et al. 2002; Kirby et al. 2009; Kirby et al. 2010). New investigations of screening for dengue prevention are also underway. Recent studies in a high-risk dengue setting in Mexico have shown window and door screens to be a popular and widely-adopted intervention that can significantly reduce domestic infestations of *Aedes aegypti* (Manrique-Saide et al. 2015; Jones et al. 2014). Given the weight of evidence to date, it is clear that house screening should be evaluated in a randomised controlled trial to fully determine the protective effect of house screening against dengue.

Evidence for the impact of community-based campaigns on dengue incidence came in the form of a multi-pronged environmental management project (14) (Figure 3.5) and the use of the larvicide pyriproxifen (36), wherein the odds and rate of dengue incidence were significantly reduced respectively. Clearly, the presence of only two non-randomised studies is not enough to dispel or corroborate dichotomous views on the subject of community-based interventions, especially as there were multiple interventions present in the former particular study; yet, perhaps this result should be taken in the context of other study designs that examined the impact of community-based campaigns on vector indices. Three randomised controlled study designs demonstrated an impact on mosquito metrics (47,29,39) (Figure 3.7), and while there was no singly evaluated intervention among these studies, this should not detract from the overall positive result. Coupled with supporting evidence from a controlled trial that also demonstrated a reduction in the House Index (4) (Figure 3.11), the evidence mounts behind a largely positive view on community-based campaigns. Recently published data also corroborate these findings – community augmentation of existing vector control techniques demonstrated a dramatic reduction in both the vector indices and dengue incidence in a cluster randomised controlled trial (Andersson et al. 2015). However, the community-based control of other disease vectors, such as the tsetse fly, has proven ineffective (Goutex & Sinda 1990). But rather than reflecting a failure of community-based campaigns in general, these failures likely stem from poor involvement of the community as a stakeholder in the implementation of community-based campaigns from the outset, and only highlights the need for greater community involvement of the community at the earliest stages of campaign development (Kovacic et al. 2013).

So what can the dengue community take from this evidence? Firstly, a word of caution, as the studies that demonstrated an effect on vectors reported only on immature stage *Aedes* mosquitoes, rather than the adult, excepting the two trials where the outcome was dengue incidence. Secondly, since community-based interventions typically involve multiple approaches to vector control, attributing any impact to a specific intervention is likely to be impossible. Of interest, however, are the elements in common to the studies that reported these results, which include: 1) community sensitisation and mobilization (including multidisciplinary steering committees comprising epidemiologists, entomologists, sociologists and clinicians, the creation of

‘Community Working Groups’ (CWG) and adequate training for those involved); 2) creation of formal channels between CWGs and municipal vector control personnel and/or other existing health structures; 3) baseline community needs assessment by CWGs; 4) engagement and empowerment via campaigns promoting behaviour change, CWG involvement to encourage and sustain vector control practices (*e.g.* source reduction: water covers, clean-up, effective larvicidal and waste disposal activities) and clinical/ vector surveillance practice; 5) monitoring and evaluation conducted vertically and/or horizontally. Note that the principal vector control measure here, source reduction, was also successfully used during the 1960s *Ae. aegypti* elimination campaign (Brathwaite Dick et al. 2012). While it is clear that further evidence is needed to distil the truly effective components of community-based combination campaigns, nevertheless, this evidence suggests that community-led or community-augmented combination approaches are a valid and outstanding area of research.

Targeting the immature stage *Aedes* mosquito is a popular control method, as identified above. And yet, these strategies do not impact on adult mosquito longevity, which likely limits their potential to reduce dengue transmission, particularly during outbreaks - hence the need for methods that also impact adult mosquito populations. While there is an expanding publication record of mosquito traps that may be useful for surveillance (37) (SantAna et al. 2014; Eiras & Resende 2009), there remains an absence of mosquito trapping methods that can adequately control the vector. Indeed, in this review, the only study included in a meta-analysis failed to show any statistically significant effect in reducing the circulating mosquito population (Figure 13). Accordingly, this remains an area in need of further research.

Insecticide-treated materials (ITMs), such as window/ door curtains and container covers, also offer an alternative and attractive method for targeting the adult mosquito, and while they remain popular with communities, they currently lack the robustness to be truly sustainable (10,33) (Kroeger et al. 2006). Perhaps reflecting these issues, evidence for their effectiveness was mixed: pooled results for the impact of insecticide treated curtains showed no statistically significant effect of the intervention on mosquito indices (32,33). Another that did not provide sufficient data for a meta-analysis also showed no significant difference in dengue prevalence between intervention clusters using insecticide treated curtains and control clusters (31). Yet, there is some evidence to support the use of water covers as they were successful at reducing vector metrics in one study (10), and they also featured in many successful community-based combination campaigns (4,29,38) (Vanlerberghe et al. 2011), although it is difficult to ascertain what level of impact they had.

ITMs are likely to achieve impact only in certain circumstances: where houses are categorised by fewer and smaller windows and doors (31-33) (Kroeger et al. 2006); where container productivity is particularly high amongst an identifiable container type (Manrique-Saide et al. 2011; Manrique-Saide et al. 2008); and where coverage of the intervention is particularly high (33), as seen with bed nets for malaria (Larsen et al. 2014). Equally, as these products tend to degrade over time, both physically and biochemically, unsustainable coverage will likely hinder long-term vector control (Vanlerberghe et al. 2011). At the same time, few trials have investigated the effect of ITMs on dengue incidence, thus more research is needed before entirely ruling out this approach.

Fogging/ space-spraying is a notable tool that has long been at the forefront of dengue control, which similarly targets the adult vector. It is deployed particularly during outbreaks for this very reason. Analysed in only one meta-analysis, outdoor fogging was able to positively and significantly impact the circulating mosquito population, albeit under night-time conditions (30). Given the widespread use of fogging, it is surprising that no further studies have been conducted on this well-known intervention. Indeed, no randomised controlled trials have been undertaken to evaluate the effectiveness of space-spraying or fogging for reducing dengue transmission or dengue incidence, anywhere in the past 35 years. Literature reviews preceding this systematic review, that included reports published before 1980, also noted this serious omission (Erlanger et al. 2008; Esu et al. 2010). Still, in theory, this approach has the particular advantage of rapidly reducing the mean age of the circulating mosquito population - a result that targeting immature stage mosquitoes cannot currently achieve – and for this reason, should not be discounted until evidence is found to the contrary. Indeed, it was also used to great effect during the 1960s *Ae. aegypti* eradication effort (Soper 1967). However, now that dengue is present in 4 continents, with concomitant transmission of multiple serotypes (Messina et al. 2014), global dengue epidemiology is all but unrecognisable to that period, rendering mass fogging campaigns financially unsustainable and operationally unrealistic. For this reason, compartmentalised use is recommended and indeed remains a popular strategy during outbreaks. And yet, demonstration of impact on vector populations is no guarantee that fogging, or indeed any other intervention, will translate into a reduction in dengue transmission. This is true for any vector-borne disease (Wilson et al. 2014) but perhaps even more so for dengue, where the indices used to measure domestic infestation rates are not accurate indicators of mosquito abundance at the time of sampling, nor are they reliable indicators of transmission risk spatially, since infective bites occur during the day, when many humans spend the majority of their time at locations far from the home (Stoddard et al. 2013; Stoddard et al. 2009). For the reasons outlined above and the absence of reliable evidence from even one randomised controlled trial, further research in this area is desperately needed.

Other potential methods available for impacting the adult mosquito include indoor residual spraying and genetic control. Two observational studies reported on the impact of IRS, and while one of these reported a positive significant reduction in the odds of (secondary) incidence (22), the second study reported an insignificant increase (25). Consequently, the pooled odds ratio showed no statistically significant effect between intervention and control groups. While indoor residual spraying can target *Aedes aegypti*, such methods have rarely been used, nor are currently recommended (Giglioli 1948; Nathan & Giglioli 1982; Doke et al. 2000; World Health Organisation 2006). Yet IRS is already used widely to control a number of other vector-borne diseases in various settings worldwide (N'Guessan et al. 2010; Picado et al. 2010). As it allows the delivery of a range of different insecticide classes, it can be an important tool for managing insecticide resistance (Kelly-Hope et al. 2008). The possibility that existing IRS programs might be expanded with minimal change to include dengue is an attractive prospect.

One study evaluating the effectiveness of novel genetic methods for control of *Aedes spp.* demonstrated a significant reduction on the circulating mosquito population. As a particularly contemporary avenue of research, the recent literature is rich with publications describing the development of these novel methods, and also provides evidence of their dramatic performance in field conditions (85) (Hoffmann et al. 2011; Moreira et al. 2009). Given the speed with which this field has developed, and the relatively high number of successes shown in recent years, many countries are willing or eager to test these methods as early as possible (Maciel-de-Freitas et al. 2012). To ensure that these potentially revolutionary approaches are utilised to best effect, considerable efforts to maintain inclusivity and transparency of such trials within vulnerable communities should be continued, or risk hindering the prospects of these approaches (Reeves et al. 2012; Lehane & Aksoy 2012; Wolbers et al. 2012; McNaughton & Duong 2014; Ramsey et al. 2014).

There was no evidence to demonstrate any impact of mosquito repellents (8), insecticide-treated bed nets (8,25) or mosquito traps (25) on the odds of dengue incidence. Another review recently reported that there was no evidence that skin repellents were beneficial in preventing malaria (Wilson et al. 2014). However, a new generation of repellents is being investigated at present, with a view to deployment within houses to prevent entry, possibly in combination with attractant lethal traps located outside in what is termed a 'push-pull' strategy (Achee et al. 2012). Initial studies indicate that these methods have greater potential than prior technologies, and at insecticide levels far lower than those necessary to kill mosquitoes (Achee et al. 2012). These trials will hopefully supersede evidence found in this review, in particular the significant associations found between the use of insecticide aerosols (8), mosquito coils (8,25) and higher odds of dengue incidence. Of course, one or two factors could explain these observations: 1) use of these items increased in response to public alerts about dengue, and actual or perceived increases in mosquito numbers during periods of dengue transmission; 2) householders using aerosols or coils might not have adopted other preventative measures, which might otherwise have decreased the incidence of dengue among this group.

Finally, three studies evaluated the use of natural predators of mosquito immature stages as biological control agents, all of which involved copepods (aquatic Crustaceans) but none of these provided sufficient data to be included in meta-analyses (34,35) (Kay & Nam 2005).

Randomized controlled trials are the most robust design for evaluating effectiveness of any intervention (Chan 2003). Of the 19 studies included for meta-analysis, only 8 (7 CRCTs, 1 RCT) were randomised. Notably, of the 8 studies that reported a positive reduction in dengue incidence at $p<0.05$, none of these results were among randomised studies (Figure 3.3). Such data highlight two important details: that achieving a reduction in dengue incidence within the stringent confines of randomised study designs is difficult, probably due to the multitude of factors that contribute to dengue transmission (Campbell et al. 2013), and/ or that possible sources of confounding and bias creep into other study designs, which result in the apparent causal associations between intervention and outcome. For all outcomes evaluated in this review, randomised controlled trial data are still desperately needed.

In total, 23/41 studies examined the impact of insecticide-based tools, yet only 9 of these cited recent insecticide resistance evidence, or indeed conducted an evaluation of the susceptibility status of the target vector population at any stage of the study. Resistance to pyrethroids and other recommended insecticides has been well documented and continues to spread among numerous vectors, including those of dengue (Ranson et al. 2010). Indeed, such is the scale of the problem that insecticide-based interventions are diminishing in effectiveness in dengue-endemic locations (Grisales et al. 2013). Clearly, insecticide susceptibility testing must be an integral part of any trial where insecticide-based interventions are under evaluation, as recommended by the World Health Organisation (World Health Organisation 2012a).

Today, a common view of dengue vector control is that existing methods are not effective and will not reduce dengue transmission (Maciel-de-Freitas et al. 2012; Maciel-de-Freitas & Valle 2014; Paul et al. 2014). We argue that our systematic review has demonstrated quite the contrary. There is evidence for the success of community-based combination campaigns on vectors, and suggestive evidence that this translates into an impact on dengue transmission. Indeed, there also exists evidence, albeit weaker, that house screening holds the potential for long-term sustainable reductions in dengue burdens. Finally, fogging and genetic methods as a means of dengue outbreak control remain inconclusive, if cautiously positive. Clearly, there is an urgent need for undertaking further work.

LIMITATIONS

Review Limitations

Descriptive statistics, such as self-reported reductions in the outcome where $p<0.05$, are exactly that. No further analyses were conducted on these data and they should be considered in the light of any potential publication bias.

Only the metrics generated when intervention coverage was highest were used for resulting meta-analyses, as these better represent the true effect of the intervention in question.

The authors acknowledge the value of reported data on the uptake of knowledge in relation to dengue and associated interventions. However, these data were not explored as a consequence of strict inclusion/ exclusion criteria.

Sensitivity analyses and funnel plots had a planned use within the review. However, due to the low number of studies available for each forest plot, any results were deemed inappropriate and thus discarded.

CONCLUSIONS & RECOMMENDATIONS

- Further evaluative work must be undertaken to explore the link between intervention and impact on vector indices and subsequent risk of dengue transmission. Generally, outcomes should comprise dengue incidence and at least one vector metric, preferably a measure of adult mosquitoes, so that clearer associations between vector control and dengue incidence can be established.

- The intervention outputs used in each meta-analysis have been assessed using a random effects model, which accounts for the likely variation observed between contexts. For example, there may be differences in seropositivity rates, population demographics and house structure. This implies that intervention success is, to some extent, context dependent.
- Mixed evidence of the effect of IRS and ITMs cannot support its use as a first line defence against dengue.
- Many studies predate present-day resistance levels. New trials that incorporate careful resistance monitoring are needed.
- Randomised study designs were relatively few in number (8/19 included in meta-analyses), indicating a need for an increase in such ‘gold standard’ trials. However, this likely also reflects the difficulty in conducting CRCT/ RCTs and alludes to the reoccurring problem of contamination of control sites due to existing public sector vector control programs, invasion of mosquitoes from neighbouring control sites and word-of-mouth dissemination of interventions into neighbouring clusters.
- Mosquito coils, repellents, bed nets and traps did not evidence a reduction in dengue incidence thus are not recommended.
- Suggestive evidence supports the use of water covers as a means to reduce mosquito indices, although these were often used in combination with other community-based interventions.
- Limited evidence suggests that fogging and genetic methods (RIDL) can impact vector indices, and given that this directly influences the mean age of circulating adult mosquito populations, is a reasoned response during outbreaks. However, no evidence indicates that this translates into a relative reduction in dengue incidence. Further trials are desperately needed in order to validate this as an effective method for controlling dengue outbreaks.
- House screening is recommended for further evaluation, as evidence suggests it may be effective in sustainably reducing dengue incidence
- Community-based/ augmented combination vector control campaigns can synergistically impact vector indices – a growing body of evidence suggests that it is also effective at reducing dengue incidence.

Included Study References

1. Sanchez, L. et al., 2008. A community education strategy to promote participation in dengue prevention in Cuba. *Revisista de Pan Americana Salud Pública,* 24(1), pp.61–9.
2. Hanna, J.N. et al., 2001. An epidemic of dengue 3 in far north Queensland, 1997-1999. *Medical Journal of Australia*, 174(4), pp.178-82.
3. Vanlerberghe, V. et al., 2010. Community involvement in dengue vector control: Cluster randomised trial (Reprinted from Brit Med J. vol 338, b1959, 2009). *Medic Review*, 12(1), pp.41-7.
4. Baly, A. et al., 2009. Cost-effectiveness of a community-based approach intertwined with a vertical Aedes control program. *American Journal of Tropical Medicine & Hygiene*, 81(1), pp.88-93.
5. Victor, T.J. et al., 2002. Dengue fever outbreaks in two villages of Dharmapuri district in Tamil Nadu. *Indian Journal of Medical Research*, 30(116), pp.133-9.
6. Wang, N.C., 1994. Control of dengue vectors in Singapore. *Kaosung Journal of Medical Sciences*, 1(10), pp.S33–8.
7. Morens, D.M. et al., 1986. Dengue in Puerto Rico, 1977: public health response to characterize and control an epidemic of multiple serotypes. *American Journal of Tropical Medicine & Hygiene*, 35(1), pp.197–211.
8. McBride, W.J. et al., 1998. Determinants of dengue 2 infection among residents of Charters Towers, Queensland, Australia. *American Journal of Epidemiology*, 148(11), pp.1111-6.
9. Swaddiwudhipong, W. et al., 1992. Effect of health education on community participation in control of dengue hemorrhagic fever in an urban area of Thailand. *South East Asian Journal of Tropical Medicine & Public Health*, 23(2), pp.200-6.
10. Kusumawathie, P.H.D. et al., 2009. Effectiveness of net covers on water storage tanks for the control of dengue vectors in Sri Lanka. *Journal of Vector Borne Diseases*, 46(2), pp.160-3.
11. Gurtler, R.E., Garelli, F.M. & Coto, H.D. Effects of a five-year citywide intervention program to control Aedes aegypti and prevent dengue outbreaks in northern Argentina. *PLoS Neglected Tropical Diseases*, 3(4), pp.e427.
12. Ávila Montes, G.A. et al., 2004. Evaluation of an educational module on dengue and Aedes aegypti for schoolchildren in Honduras. *Revista de Pan Americana Salud Pública*, 16(2), pp.84–94.
13. Pessanha, J.E.M. et al., 2009. Evaluation of the Brazilian national dengue control plan. *Cadernos do Saude Publica,* 25(7), pp.1637-41.
14. Toledo, M.E. et al., 2011. Evidence on impact of community-based environmental management on dengue transmission in Santiago de Cuba. *Tropical Medicine & International Health,* 16(6), pp.744-7.
15. Murray-Smith, S., Weinstein, P. & Skelly, C., 1996. Field epidemiology of an outbreak of dengue fever in Charters Towers, Queensland: are insect screens protective? *Australia & New Zealand Journal of Public Health*, 20(5), pp.545–7.
16. Omar, M. et al., 2011. Field evaluation on the effectiveness of a modified approach of chemical fogging against the conventional fogging in controlling dengue outbreak. Malaysian Journal of Pathology, 33(2), pp.113-7.

17. Pai, H.H., Hong, Y.J. & Hsu, E.L., 2006. Impact of a short-term community-based cleanliness campaign on the sources of dengue vectors: An entomological and human behavior study. *Journal of Environmental Health,* 68(6), pp.35-9.
18. Igarashi, A., 1997. Impact of dengue virus infection and its control. *FEMS Immunology & Medical Microbiology*, 1997. 18(4), pp.291-300.
19. Sanchez, L. et al., 2009. Intersectoral coordination, community empowerment and dengue prevention: six years of controlled interventions in Playa Municipality, Havana, Cuba. *Tropical Medicine & International Health*, 14(11), pp.1356-64.
20. Pinho, S.T. et al., 2010. Modelling the dynamics of dengue real epidemics. *Philosophical Transactions Series A*, 368(1933), pp.5679-93.
21. Huy, R. et al., 2010. National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control. *Bulletin World Health Organisation*, 88(9), pp.650–7.
22. Vazquez-Prokopec, G.M. et al., 2010. Quantifying the spatial dimension of dengue virus epidemic spread within a tropical urban environment. *PLoS Neglected Tropical Diseases*, 4(12), pp.e920.
23. Lin, T.H., 1994. Surveillance and control of Aedes aegypti in epidemic areas of Taiwan. *Kaosung Journal of Medical Sciences,* 10, pp.S88-93.
24. Lloyd, L.S. et al., 1994. The design of a community-based health education intervention for the control of Aedes aegypti. *American Journal of Tropical Medicine & Hygiene*, 50(4), 401-11.
25. Ko, Y.C., Chen, M.J. & Yeh, S.M., 1992. The predisposing and protective factors against dengue virus transmission by mosquito vector. *American Journal of Epidemiology*, 136(2), pp.214-20.
26. Jayasooriya, G.A. et al., 2009. Use of geographical information system (GIS) and global positioning system (GPS) for dengue and dengue haemorrhagic fever control in Sri Lanka. *Dengue Bulletin*, 33(1), pp.11-20.
27. Toledo, M.E. et al., 2007. Towards active community participation in dengue vector control: results from action research in Santiago de Cuba, Cuba. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 101(1), pp.56-63.
28. Kay, B.H.B. et al., 2002. Control of aedes vectors of dengue in three provinces of Vietnam by use of Mesocyclops (Copepoda) and community-based methods validated by entomologic, clinical, and serological surveillance. *American Journal of Tropical Medicine & Hygiene*, 66(1), pp.40-8.
29. Arunachalam, N. et al., 2012. Community-based control of Aedes aegypti by adoption of eco-health methods in Chennai City, India. *Pathogens & Global Health*, 106(8), pp.488-96.
30. Farajollahi, A. et al., 2012. Effectiveness of ultra-low volume nighttime applications of an adulticide against diurnal Aedes albopictus, a critical vector of dengue and chikungunya viruses. *PLoS One*, 7(11), pp.e49181.
31. Loroño-Pino, M.A. et al., 2013. Towards a casa segura: a consumer product study of the effect of insecticide-treated curtains on Aedes aegypti and dengue virus infections in the home. *American Journal of Tropical Medicine & Hygiene*, 89(2), pp.385-97.

32. Lenhart, A. et al., 2013. A cluster-randomized trial of insecticide-treated curtains for dengue vector control in Thailand. *American Journal of Tropical Medicine & Hygiene*, 88(2), pp.254-9.
33. Vanlerberghe, V. et al., 2013. Coverage-dependent effect of insecticide-treated curtains for dengue control in Thailand. *American Journal of Tropical Medicine & Hygiene*, 89(1), pp.93-8.
34. Nam, V.S. et al., 2012. Community-based control of Aedes aegypti by using mesocyclops in Southern Vietnam. *American Journal of Tropical Medicine & Hygiene*, 86(5), pp.850-9.
35. Vu, S.N. et al., 2005. Elimination of dengue by community programs using Mesocyclops (Copepoda) against Aedes aegypti in central Vietnam. *American Journal of Tropical Medicine & Hygiene*, 72(1), pp.67–73.
36. Ocampo, C.B. et al., 2014. Reduction in dengue cases observed during mass control of Aedes (Stegomyia) in street catch basins in an endemic urban area in Colombia. *Acta Tropica*, 132, pp.15–22.
37. Degener, C.M. et al., 2014. Evaluation of mass trapping for dengue vector control - A cluster RCT in Brazil 2014. *Journal of Medical Entomology*, 51(2), pp.408-20.
38. Tsunoda, T. et al., 2013. Field trial on a novel control method for the dengue vector, Aedes aegypti by the systematic use of Olyset. *Parasites & Vectors,* 6(1), pp.1-1.
39. Castro, M. et al., 2012. A community empowerment strategy embedded in a routine dengue vector control programme: a cluster randomised controlled trial. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 106(5), pp.315-21.
40. Stoddard, S.T. et al., 2014. Long-term and seasonal dynamics of dengue in Iquitos, Peru. *PLoS Neglected Tropical Diseases*, 8(7), pp.e3003.
41. Harris, A.F. et al., 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology*, 30(9), pp.828-30.

CHAPTER 4

EVALUTION OF VECTOR CONTROL TOOLS FOR DENGUE OUTBREAK RESPONSE: STUDY PROTOCOL

ABSTRACT

Background

The objective of this trial was to investigate whether interventions against the primary vector were able to significantly reduce circulating mosquito populations, and whether such a reduction was able to further impact the number of clinically-reported dengue cases.

Method/ Design

A cluster-randomised controlled trial study design was used. 2500 households were randomised to form clusters of 5 x 100 households, giving a total of 500 households per study arm (4 interventions and one control (C)). The unit of analysis was the cluster and eligible households were chosen from the city of Bandar Lampung, Sumatra, Indonesia. Clusters were randomised to receive one of four interventions or the control (routine measures). The primary outcome was chosen as the *Stegomyia* indices and mosquito adult catches, with secondary outcomes comprising clinically reported dengue incidence and household acceptability of the intervention(s).

Discussion

This is the first cRCT to investigate the impact of Do-It-Yourself (DIY) indoor residual spraying using handheld spray cans on vector abundance, alongside the implementation of household GPS-linked questionnaire/ vector data.

INTRODUCTION

Vector control is currently the only approach available to limit dengue transmission (Achee et al. 2015). Even with the advent of a successful tetravalent vaccine, it is likely that combined vaccine and vector control use will persist (Andraud et al. 2012). In light of this, empirical evidence is necessary to evaluate the impact of existing and novel vector control tools.

While there are many approaches to dengue vector control, fogging is arguably the most widespread, especially during outbreaks (Harrington et al. 2013). Despite this, little is known of its effectiveness (Esu et al. 2010). And while fogging may potentially impact vector abundance over the short term, there are alternate existing strategies, such as indoor residual spraying, already proven effective for other endophilic mosquitoes (N'Guessan et al. 2010), that could provide a longer-term reduction in vector abundance. Indeed IRS has been the predominant approach used against the vector Triatomine bugs for the disruption of chagas transmission, and has succeeded in dramatically reducing vector prevalence (Lehane & Aksoy 2012). In addition, residual spraying has also been successful in reducing malaria transmission by targeting the *Anopheles* mosquito vector (Pluess 2013; Kim et al. 2012). As discussed in Chapter 1, the use of IRS might yet be an effective approach to reduce the abundance of adult dengue vectors. Yet, in spite of such promise, few RCTs have assessed its effectiveness against *Ae. aegypti* (Esu et al. 2010).

In contrast to the vertical approaches described above, horizontal approaches, or community-based interventions, may augment the effectiveness of vertical vector control tools by empowering community members to take decisions that directly affect their health leading to improved deployment and practice (Lloyd et al. 1994). Indeed over recent years, community-based interventions as a practical method for sustainable vector control are becoming increasingly adopted as studies demonstrate their effectiveness (Vanlerberghe et al. 2010; Arunachalam et al. 2012; Baly et al. 2009; Andersson et al. 2015). One widely known commercial product that combines insecticide with community empowerment is the hand-held insecticide spray can.

However, given the widespread availability of handheld spray-cans, there is surprisingly little evidence to suggest that such residual formulations impact mosquito abundance and dengue transmission.

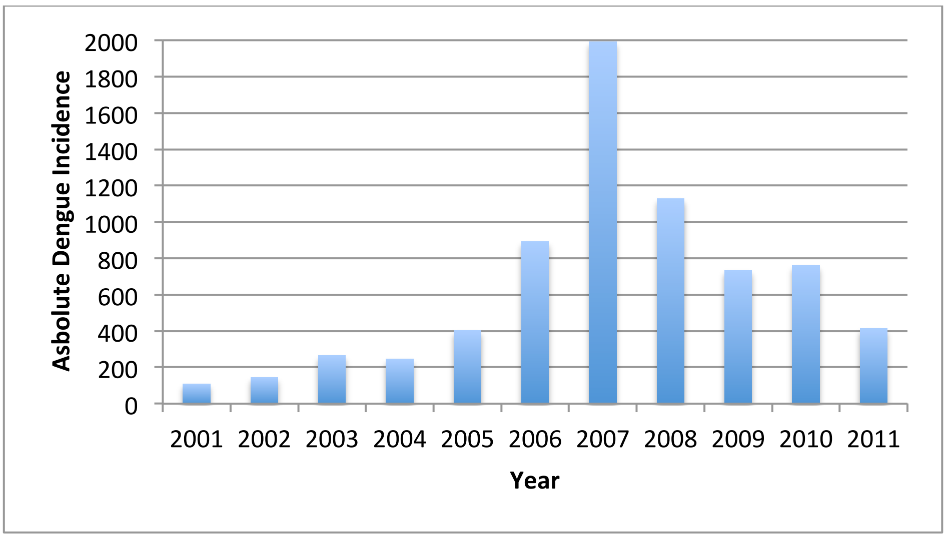
In light of the absence of evidence to support any one of these approaches, further trials are necessary to a) validate both vertical and horizontal vector control interventions as effective vector control measures and b) define the level by which mosquito abundance should be reduced in order to impact dengue transmission.

MATERIALS AND METHODS

Location

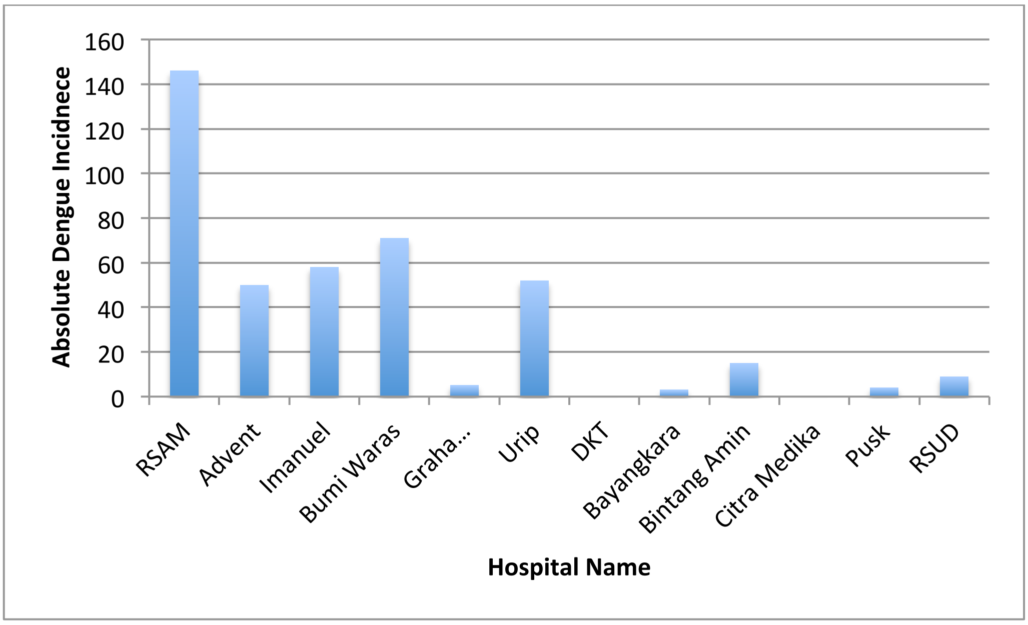
The trial site was chosen as Bandar Lampung in Sumatra, Indonesia. Situated within the province of Lampung, dengue affects all 14 municipalities (7,608,405 population), of which Bandar Lampung is one municipality comprising 13 districts, 98 sub-districts and a population of 881,801 as of 2011. Dengue incidence in 2011 was reported as highest amongst 5-14yrs (41%), followed by 15-44yrs (29%). Cases (defined as clinically diagnosed using antigen test NS1) saw a steady upward trend from years 2001 to 2005, with 2006 being considerably higher than the previous five years (892 cases or 101.1/ 100,000) (Figure 4.1).

Figure 4.1. Number of absolute dengue cases in Bandar Lampung from 2001 to 2011 (Uiskm 2012).



In 2007, national health insurance was implemented; this was free for lower socio-economic classes and coincided with the largest report of dengue cases (1992) to date (225.9/ 100,000) (Figure 4.1). Cases in 2011 have since reduced to pre-2006 levels: 413 (46.8/ 100,000). All calculations were conducted using 2011 census data. Without considering other variables that may impact these incident data, due to the relatively benign nature of the majority of dengue cases, it is likely that all incident dengue cases for each year represent an underestimate (Figure 4.2).

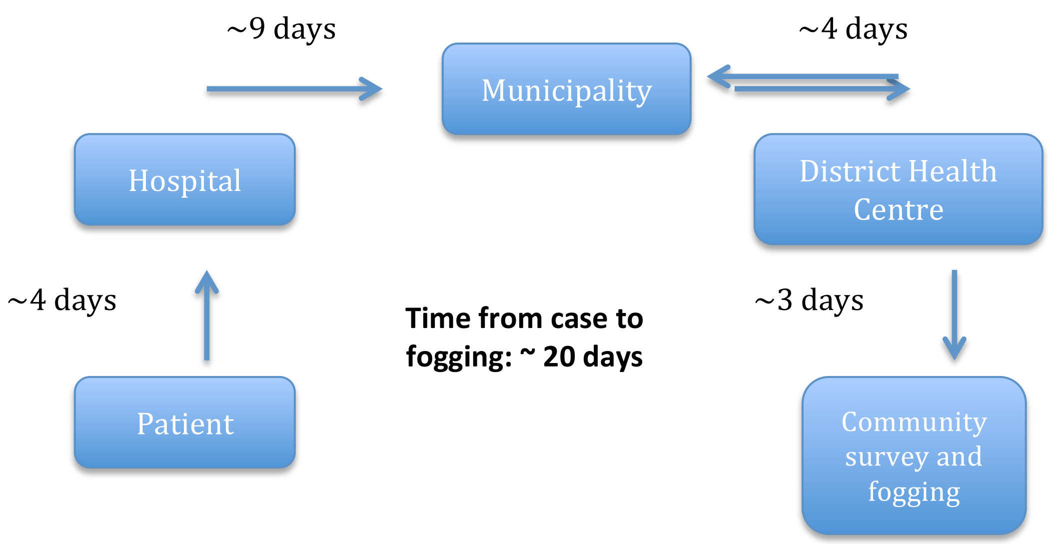
Figure 4.2. Dengue Cases in 2011 stratified by hospital in Bandar Lampung, Sumatra. Dengue was confirmed by standard WHO case definitions (World Health Organisation 2009). Dengue incidence reporting was successfully completed by 10/12 hospitals (Uiskm 2012).



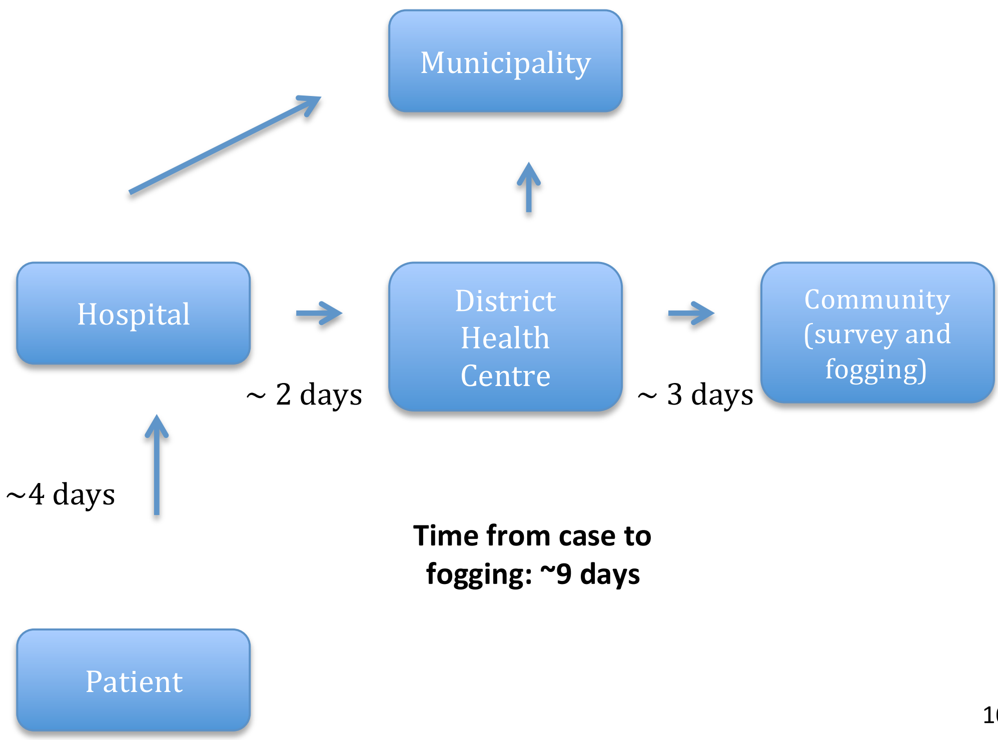
Dengue Case Reporting

There are 13 hospitals and 28 community health centres spread across 98 sub-districts within Bandar Lampung. Reporting systems for dengue cases are in place but often convoluted and untimely, especially outside 'outbreak' periods. There are three protocols for dengue reporting that predominate throughout the municipality of Bandar Lampung. In all systems, the patient can directly report to the municipality or the district health centre, unfortunately causing duplicate cases, thereby resulting in over-estimates.

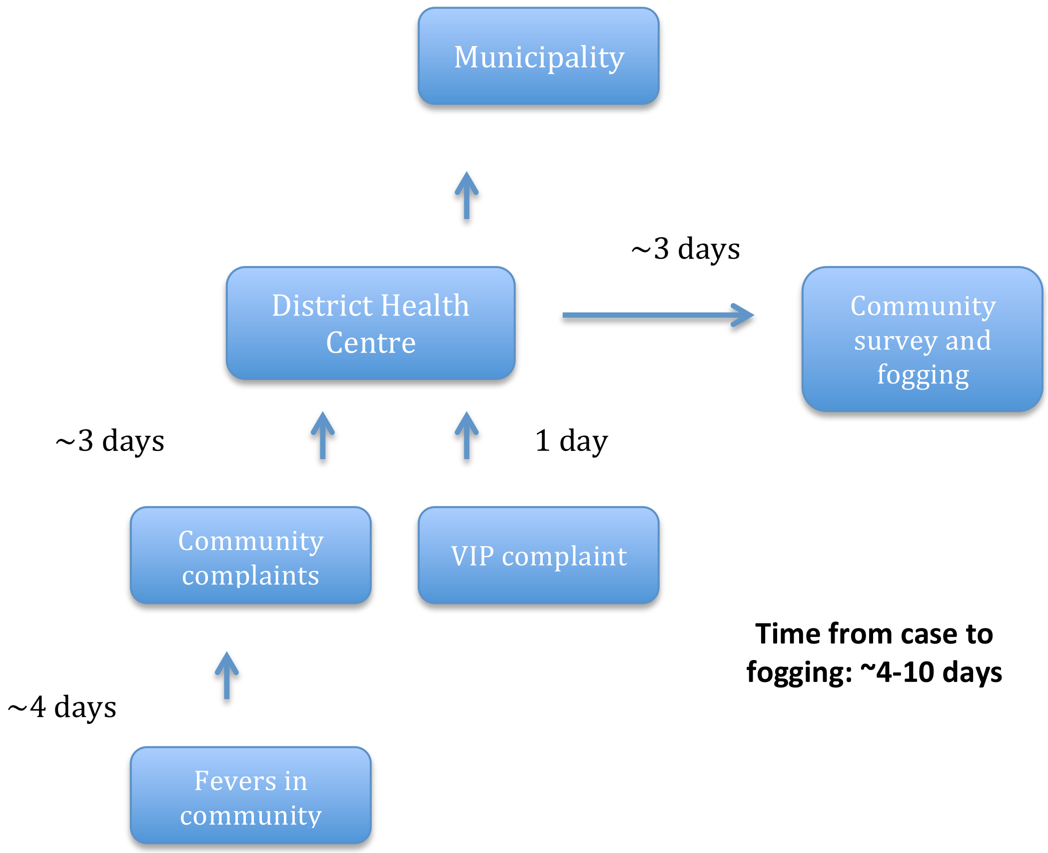
During non-outbreak periods (defined as <2-fold increase in cases during the previous month or of the same month the year prior), reporting of cases follows the diagram below:



During outbreak periods the response is as follows:



Entomological surveys and fogging are affected by direct reports from either the community or 'VIPs, which hasten the response.



These three examples identify the complex and inefficient nature of dengue response in Bandar Lampung, highlighting the need for simple and effective reporting and response systems.

Climate

The rainy season spans the period between December and March while the dry season is generally between the months of June to September (Weather Online 2015).

Objectives

This trial aimed to evaluate the impact of a number of vector control approaches on the local dengue vector populations. These were

1) Outdoor Fogging (OF)
2) Indoor Fogging (IF)
3) Indoor Residual Spraying
4) DIY IRS

The acceptability of each intervention to the community was to be assessed by a series of qualitative outputs, with data collected by questionnaire-based surveys.

Additionally, the trial aimed to monitor any potential impact of each approach on dengue incidence, by investigating clinically reported dengue cases among municipal hospitals, during the period of the trial. The study also sought to test the feasibility of utilising digital tablets to capture qualitative and quantitative data.

Interventions

Similar across much of the dengue endemic world, dengue control in Bandar Lampung is characterised by a response to reported cases using outdoor fogging. Accordingly, this trial aims to evaluate the most widely used methods of vector control to determine effectiveness against the vector and dengue transmission.

1. Outdoor fogging will be used to significantly impact numbers of circulating adult/ immature mosquitoes over the immediate short term (<1 month). Testing this intervention is crucial due to the absence of evidence for effectiveness as the predominant form of dengue outbreak response.

2. Equally, indoor fogging will be used to significantly impact the adult mosquito population over the immediate short term, however this intervention is expected to result in low user acceptability.

3. Thirdly, IRS will be used to significantly impact the number of circulating adult mosquitoes over the medium - long term (>3 months). IRS has a previous successful history with global malaria and chagas programmes and acceptability is expected to remain relatively high where the intervention is

successful over the expected term.

4. Finally, DIY IRS will be used to significantly impact the adult mosquito population over the medium term (<3 months). This approach is expected to garner high user acceptance rates and represents an attempt to quantify the effectiveness of such widely available aerosols sold and promoted via the private market.

Study Design

The study is a cluster-randomised controlled trial design, comprising 5 study arms. The intervention will be delivered at the household level to those who agree to participate in the trial. Randomised controlled trials are considered the 'gold standard' in epidemiological study design (Chan 2003). A cluster design is considered most appropriate due to the high likelihood of neighbourhood contamination from mosquito movement between households, and to ensure comparability between existing cluster-randomised controlled trials (MRC 2000).

Sample Size

Sample size calculations were carried out in STATA 13.1 (StataCorp 2013) in accordance with the methods used by Hemming *et al* (2011) (Hemming et al. 2011).
Using the household as the unit of analysis with a binomial significance test, anticipating a reduction to 50% house infestation in intervention arms using an alpha of 0.05, power of 0.8 with an average cluster size of 100 while adjusting the intra-cluster correlation (ICC) of 0.1, 5 trial arms with 500 houses per arm for a cluster randomised design was determined to be sufficient to achieve a power of 0.81. Note that the sample size per arm was rounded up and 1 extra cluster was added in case of a t distribution (non-normal distribution arising from small sample size). If a cluster were to be lost and not replaced in any arm, the power would be reduced to 0.69.

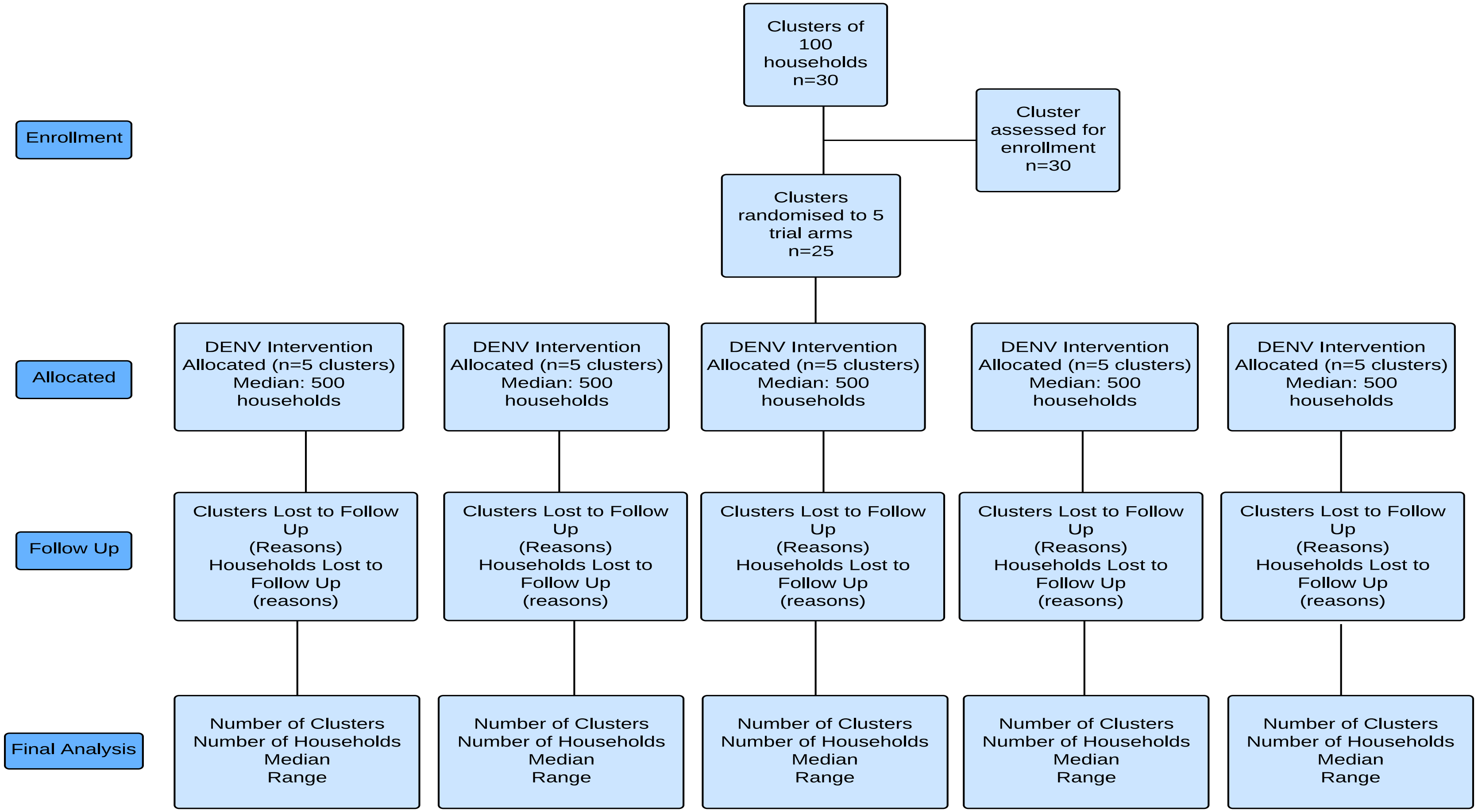
Location and Recruitment of Households

Visits to various areas of Bandar Lampung prior to the project determined possible study sites based on the attributes listed below. Clusters of 100 households were designated using panchromatic images (2006) provided by WorldSat International (WorldSat International 2012) and were chosen from within areas of Bandar Lampung where:

1) Annual dengue incidence was high
2) Socio-economic status was similarly low
3) House structure generally comprised open eaves with a distinct absence of air conditioning units
4) Community health workers were present

Each cluster was randomly assigned to a trial arm (DIY-IRS, IRS, OF, IF, C) using a random number generator (Figure 4.3). Clusters were spatially distinct from one another, with a minimum of >200m between neighbouring clusters, as this is the accepted flight range of *Ae. aegypti* (Harrington et al. 2005). Finally, a total of 30 viable clusters were identified in case of significant loss to follow up, or poor enrolment rates, necessitating the inclusion of another cluster.

Figure 4.3. Flow chart of household/ cluster selection. Households selected from pre-defined randomised cluster areas in Bandar Lampung.



Household/ Cluster Exclusion

Households will be excluded where informed/ written consent is not obtained. Clusters will be disregarded where study enrolment rates fall below 75% of the necessary households for a complete cluster or loss to follow up is greater than 35%.

Insecticides

There is no widely available insecticide resistance data for Bandar Lampung yet baseline surveys aim to reveal the presence of insecticide resistance within dengue vectors.

The insecticides of choice are pyrethroids due to acceptability, efficacy and safety profiles. Consequently, outdoor fogging, indoor fogging and IRS will use the same pyrethroid: Lambdacyhalothrin (Icon; Syngenta), in one of two formulations:

1) Icon EC (emulsifiable concentrate) for outdoor fogging and indoor space spraying; diluted in diesel and delivered @ 1-2 gal/ha (outdoors) or at a fixed period of 30-40 secs discharge/room (indoors)
2) Icon CS (capsule suspension) for indoor residual spraying; this microencapsulated formulation has improved lifespan and therefore extends the duration of effect after a single treatment; application rate of 20-30mg a.i./m^2

Lambdacyhalothrin has a particularly good safety profile (class 3 WHO hazard category) and has been rigorously tested and evaluated by WHOPES as an acceptable component of indoor residual spraying for malaria prevention and control (World Health Organisation 2015b). Both EC and CS formulations are licensed for use in Indonesia.

DIY-IRS Baygon handheld products will be used instead of lambdacyhalothrin due to supply issues with the originally planned Syngeta products. Nonetheless, Baygon still utilises pyrethroid insecticides and will be acceptable based on the formulation that contains alpha-cypermethrin (0.1%), prallethrin (0.03%) and imiprothrin (0.031%).

Control clusters will not receive any experimental intervention yet will remain eligible to receive routine control measures (outdoor/ indoor fogging, clean up campaigns, advocacy), that will be documented spatially and temporally for subsequent data analyses.

Outcomes

The primary outcomes comprise a number of entomological indices, namely the Stegomyia indices and mosquito adults.

The secondary outcomes comprise dengue incidence, as reported by local municipal hospitals, in accordance with standard WHO dengue case definitions (World Health Organisation 2011). In addition, knowledge, attitudes and practices questionnaires will be delivered at baseline, follow up 1 and follow up 3, to gauge knowledge of dengue transmission and acceptance of each intervention.

Date Collection Methods

Each household will be inspected for the presence of larvae/ pupae in all indoor water-filled containers. Containers will be classified by container type (volumetric size and usage), position (indoors/ outdoors) and presence of larvicide.

Any uncovered household wells will also be sampled using funnel traps (Focks 2004) that will be left at the household for one week, before technicians will return to collect and quantify the catch.

Recently, novel technologies to increase the capture rate of live adult mosquitoes have emerged in the form of the Prokopack Aspirator (Vazquez-Prokopec et al. 2009). This device will be used to aspirate adult mosquitoes from all rooms of a household. Once captured, adult mosquitoes will be transferred to cup and labelled ready for transportation and subsequent identification in the laboratory.

Global positioning systems (GPS) are becoming increasingly widespread across many industries and can be linked to data capture in real-time. To ensure that household recruitment remains within the defined cluster, and to link KAP outcomes to each household, the study will utilise Google Nexus 7 tablets and DroidSurvey software (https://www.harvestyourdata.com) (Appendix 8). Data will be reviewed on a daily basis to guide future household enrolment and as a proxy quality assurance method, given that the tablet will record the number of householder KAP surveys completed, including their location, during any given day.

Coverage

Within the respective clusters, outdoor fogging will be applied until complete coverage of a cluster is achieved, while indoor fogging and IRS will be applied once per household. DIY-IRS bottles will be delivered to households for 2 complete applications within the household, specifically targeting bedrooms, bathrooms and then living areas, namely spraying corners, dark areas and behind furniture (the established resting places of mosquitoes), once at week 1 and again at week 4.

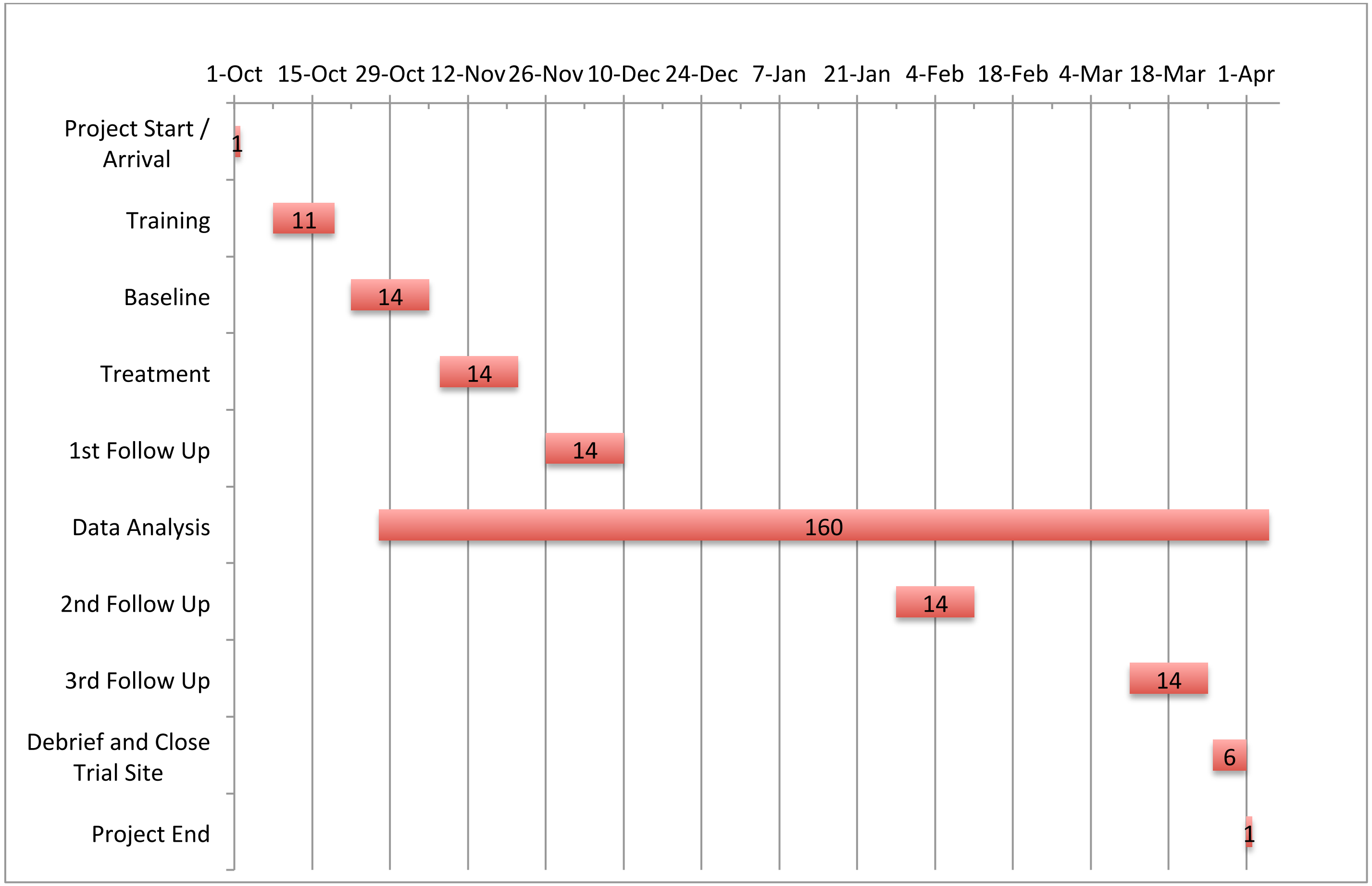
Timeframe

It is expected that the entire field trial will take no longer than 6 months (Figure 4.4). Baseline will take place over 14 days, with treatment, and three follow up periods also taking 14 days. We plan to train 120 people (60 students/ community health workers and 60 civil servants) to ensure that we have at least 40 people working at any one time based on a given skill set. Civil servants will be employed solely for treatment and students/ CHWs will be employed for all remaining stages. The following calculation provides evidence that one phase (baseline/ treatment etc.) should take no more than an estimated 2 working weeks to ensure complete coverage of the trial area:

- 40 people (20 teams) = 10-12 houses per team per day. 12*(40/2)=240 houses per day
- The above is based on a team taking 20 minutes per household and working a 5-

hour day (including one hour for lunch) during a 6-day week = 1440 household per week

Figure 4.4. Gantt Chart. Timeline of events for cluster-randomised controlled trial in Bandar Lampung, Sumatra, Indonesia.



Ethical Review

The Liverpool School of Tropical Medicine has granted ethical permission.

DISCUSSION

This is the first cRCT to investigate the impact of Do-It-Yourself indoor residual spraying on vector abundance, and the first to link tablet-captured, GPS-linked spatial, vector and KAP data.

The rationale behind these approaches is motivated by a number of factors:

1) Empowering communities to control both nuisance and disease vectors autonomously should increase intervention acceptability, thereby reducing one of the barriers to behaviour change and improving uptake (Lloyd 1994).
2) Questionnaire data are routinely captured using paper-based methodologies world over. The use of paper reduces efficiency with data collection methods and may introduce bias (Litchfield 2005). Digital technologies are generally favoured, limit data loss and may reduce potential biases (Litchfield 2005).
3) Spatial data are absent in many vector-monitoring studies – the use of GPS to link both vector and KAP data will provide unparalleled access to dengue spatial transmission dynamics.
4) Recording the spatial location of houses by GPS should minimise loss to follow up attributed to poor mapping skills.

This trial will validate these technologies and report on their use singly and in combination for future application within the fields of dengue, vector-borne disease and public health in a bid to bridge the gap between traditional and modern novel data capture methods.

Progress

The trial was completed in July 2013 but analysis and publication of the data were not permitted (Appendix 9).

CHAPTER 5

ALARM SIGNALS FOR DENGUE OUTBREAKS: A MULTI-CENTRE STUDY IN ASIA AND LATIN AMERICA

The results from this chapter have been submitted in a manuscript to PLOS Neglected Tropical Diseases.

ABSTRACT

Background

Dengue is an unrelenting economic burden worldwide, with increasingly frequent outbreaks putting pressure on already limited health infrastructure and capacity. Early warning models could allow health systems and vector control programmes to respond more effectively to mitigate the impact of dengue outbreaks.

Methodology/Principal Findings

The Shewhart method and Endemic Channel were used to identify predictors for dengue outbreaks. Five country datasets were compiled by epidemiological week and alarm/ outbreak indicators were analysed using logistic regression during the historic period (2007 – 2011). Alarm periods and outbreak periods were formed during the evaluation period (2012 - 2013). Alarm periods were thereafter used to predict outbreak periods - the success of this process was calculated using sensitivity and positive predictive value. Across Mexico and Dominican Republic, an increase in probable cases predicted outbreaks with sensitivities and positive predictive values (PPV) of 92% and 45%, and 95% and 48% respectively, at a lag of 1-12 weeks. An increase in mean temperature predicted outbreaks of hospitalised cases in Mexico, with 86% sensitivity and 44% PPV, also at a lag of 1 – 12 weeks. At a lag of 4-16 weeks, rainfall was less sensitive across some countries, and best in Brazil and Mexico, where sensitivities were 65% and 67% respectively. Relative humidity predicted with high sensitivity 88% of outbreaks in Brazil at a lag of 2-12 weeks, but was less strongly associated elsewhere.

Conclusions/Significance

The Shewhart method was able to sensitively predict dengue outbreaks using an increase in probable cases and mean temperature, and to a lesser extent, weekly rainfall and relative humidity. While the predictive capacity of these variables is context-dependent, nevertheless they should be routinely captured and utilised to warn of forthcoming outbreaks as part of a multivariate early warning system

INTRODUCTION

Dengue outbreaks can exert large pressures on public health systems, as hospitals and outpatient clinics become overwhelmed by the surge in cases, both actual and suspected (Badurdeen et al. 2013; Simmons et al. 2012). These pressures are compounded by resource-limited or weak surveillance systems that might have given prior warning if sufficient funding, expertise and methodologies were in place (Gluskin et al. 2014; Madoff et al. 2011; Runge-Ranzinger et al. 2014; Beatty et al. 2010). The ability to predict outbreaks well in advance should enable public health systems to respond more efficiently through the timely allocation of resources (Ellis et al. 2011; Badurdeen et al. 2013; Racloz et al. 2012). It is in this capacity that infectious disease modelling has become increasingly relevant (Rigau-Pérez et al. 1999; Ellis et al. 2011; Barbazan et al. 2002; Thai & Anders 2011).

To date, epidemiological variables, such as the historic incident mean plus 2 standard deviations (SD), have been used in dengue forecasting models, with some success (Phung et al. 2015; Hii et al. 2012a; Hii et al. 2012b; Hii et al. 2009). Regression functions also feature and are used to calculate the probability of an outbreak, as reported recently in Viet Nam (Phung et al. 2015) and Singapore (Xu et al. 2014). These analyses identified clear trends between abnormal changes in meteorological and/ or epidemiological variables and subsequent dengue outbreaks.

Yet vector-borne disease prediction can be variable in nature, as interactions between vector, pathogen and human are intricate and complex (Campbell et al. 2013). In particular, models struggle to accurately capture spatial and temporal data about immature life stage abundance and the prevailing number of breeding habitats (Favier et al. 2005). And while predictive models exist, these tend to focus on smaller spatial units, which are often inadequate for the district or country level responses required for public health control responses (Ninphanomchai et al. 2014; Hii et al. 2009; Hii, Zhu, et al. 2012a; Hii et al. 2012b). Programme managers and regional epidemiologists alike need user-friendly predictive models that can adequately identify inter-district dengue variation (Johansson et al. 2009; Racloz et al. 2012). Novel approaches are required to develop predictive, accessible methodologies that utilise a multitude of alarm indicators on broad spatial scales (Racloz et al. 2012). To address this, we considered the Shewhart Method.

The Shewhart Method is typically used to monitor the quality control of goods within the manufacturing process (Shewhart 1931). This method involves the use of control charts to define 'in-control' and 'out-of-control' manufacturing states, using the historic mean and standard deviation of the outcome variable (Reid & Sanders 2004; Shewhart 1931). Within a dataset, this method can identify variation that is beyond the influence of natural, random fluctuation, *i.e.* the consequence of an identifiable or 'attributable' cause or change in the process (Reid & Sanders 2004; Rigau-Pérez et al. 1999; Stroup et al. 1989). Since regional epidemiologists often collect historical data to calculate the moving incident mean (or median), applying this approach to infectious diseases modelling becomes possible. These data can then be used to forecast changes in the variable of interest, which is the primary basis of the Endemic Channel calculation (Cullen et al. 1984). In this sense, the Endemic Channel represents the number of cases within the expected normal range, or the 'in control' state, while anything above this moving threshold would be considered to represent an unprecedented number of cases and an 'out of control' state *i.e.* an outbreak. This approach is favoured in many countries, as it allows programme managers to easily define the presence/ absence of an outbreak (Badurdeen et al. 2013; Runge-Ranzinger et al. 2014; Runge-Ranzinger et al. 2008), despite the limitations associated with abnormally high historic means, and variation in the seasonal timing of dengue cases (Badurdeen et al. 2013). Such predictive methodologies have demonstrated success in both Puerto Rico and Thailand (Rigau-Pérez et al. 1999; Barbazan et al. 2002; Ninphanomchai et al. 2014), where measuring a prior increase in the outcome variable enabled models to retrospectively predict subsequent outbreak periods, thus indicating potential in prospective operational capacities. Extending this rationale further, it should be possible to investigate a preceding rise in meteorological, entomological and epidemiological independent variables (alarm indicators) to predict dengue outbreaks.

In spite of the progress made in the field of infectious disease outbreak prediction in modelling high risk areas and population dynamics (Racloz et al. 2012; Kuhn et al. 2005; Stoddard et al. 2009; Stoddard et al. 2013), reliable, affordable and practical dengue warning systems are still needed to mitigate the growing economic and human costs of dengue (Johansson et al. 2009). Accordingly, as part of IDAMS (International Research Consortium on Dengue Risk Assessment, Management and Surveillance) and WHO-TDR (World Health Organisation - the Special Programme for Research and Training in Tropical Diseases), this paper describes the development and testing of predictive methodologies based on retrospective datasets obtained from five countries in Asia and Latin America.

METHODS

Objectives

To produce a model that will enable the prediction of 'out of control' dengue cases (outbreaks) as defined by dengue incidence (probable/ hospitalised cases (World Health Organisation 2009)) using the presence of preceding 'alarm signals', defined as abnormal changes within various entomological, meteorological and epidemiological alarm indicators.

Data Collection

The five countries (Brazil, Dominican Republic, Mexico, Malaysia and Viet Nam) selected for the study were chosen from a larger group whose dengue surveillance systems had been analysed previously (Badurdeen et al. 2013; Harrington et al. 2013). Using a set of potential alarm signals for dengue outbreaks, identified by systematic literature search (Runge-Ranzinger et al. 2014) and an international expert colloquium (Badurdeen et al. 2013), a protocol for data capture based on a common data collection matrix was agreed. Retrospective data collection was conducted from October 2013 to April 2014. Data from 5 to 7 years (2007/9 – 2013) were collected. Data were split between a period of historical analysis (2007/9 – 2011) and a period of evaluation (2012 – 2013). WHO-TDR support staff periodically visited each country to ensure that the data matrix was completed accurately and to answer any queries, as well as to verify data sources to reduce the risk of misreporting. Each visit was documented in a WHO-TDR report to communicate any limitations, biases and known problems.

Data were collected for the following variables by each country representative using a predefined data capture spreadsheet: meteorological (outdoor air temperature, rainfall, outdoor humidity); epidemiological (mean age, circulating serotype, probable dengue cases, hospitalised dengue cases); entomological (Breteau Index, House Index, Ovitrap Index (Mexico only)). The epidemiological week (Sunday to Saturday) was temporal unit of data collection, while the spatial unit was based on existing district geographical boundaries (municipality in Brazil), or the country equivalent (“locality” in Mexico). Weeks 1 and 53 were excluded due to variability in data quality. All raw data were sourced in-country with the cooperation of Ministries of Health and relevant local government officials and entered into standardised forms.

All climate data were attributed to the district of interest (or the nearest possible weather station) so as to minimise spatial bias, although this was not possible in Malaysia. Consequently, external websites Wunderground (Wunderground 2015) and Tutiempo (Tutiempo 2015) were used to augment the data collection. Where these websites did not provide sufficient data, no meteorological variables were captured (Viet Nam only). Across all countries, meteorological data were only available in a daily format; accordingly, Microsoft Excel was used to generate weekly means via Pivot Tables after cleaning the datasets. Subsequently, STATA 13.1 (Stata Corp 2013) was used to merge epidemiological, entomological and meteorological datasets. No remote sensing data were collected or used.

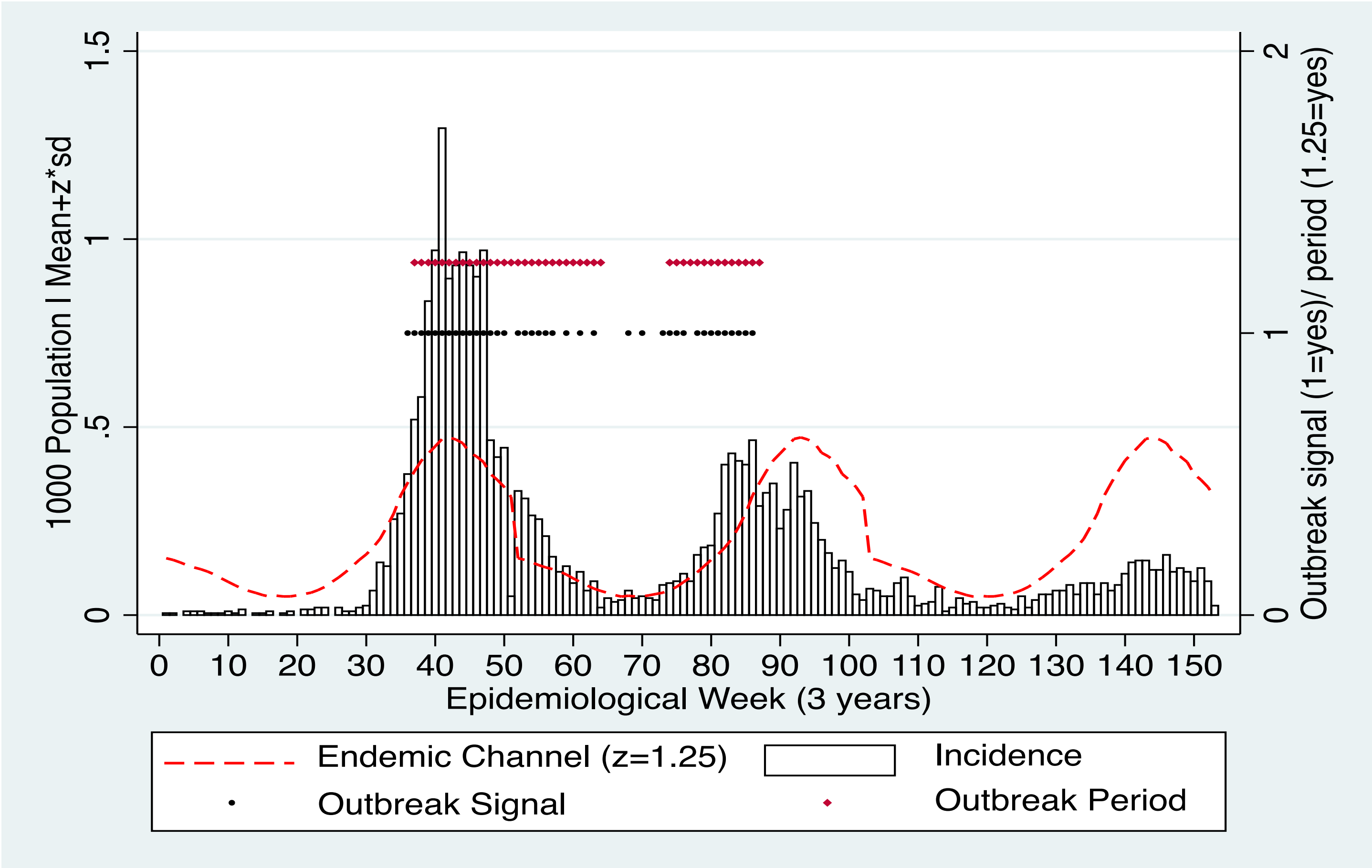
Ethical Permission

Ethical approval was sought from and granted by WHO Regional Ethical Committees in PAHO and WPRO, with the full agreement of the appropriate bodies in the participating countries.

The Endemic Channel

Calculations were performed to determine the endemic channel and thus to define outbreaks. These utilised a minimum 3-year historic period (2007/9-2011) to establish the district specific mean and standard deviation, per epidemiological week for the outcome of interest (probable and hospitalised dengue cases per district). Values were smoothed with a 13-week moving average, which included the week of observation, plus the preceding and subsequent 6 weeks (Rigau-Pérez et al. 1999; Stroup et al. 1989; Farrington & Andrews 2003). Then, for the analysis period (2012 to 2013) using a multiplier denoted 'z', it was possible to define outbreaks per district by the upper limit of the endemic channel (z times the standard deviation above the mean) (Figure 5.1).

Figure 5.1. Test Data: Outbreak signals detected where incidence crosses the Endemic Channel (z=1.25). Outbreak periods formed when 2 consecutive outbreak signals are present; outbreak periods end when 2 absent consecutive outbreak signals are registered (incidence does not cross the Endemic Channel for 2 consecutive weeks).



Without excluding any epidemic years, the moving mean captured seasonality throughout the time series. Outbreak indicators, signals and periods were defined as:

Outbreak indicator:

1) Number of weekly hospitalised cases (as defined by WHO clinical case definition/ lab confirmation) (World Health Organisation 2009) divided by the district population (hospitalised cases per 1,000 population)
2) Number of weekly probable cases (World Health Organisation 2009) divided by the district population (probable cases per 1,000 population)

Outbreak indicators with a value above the historic mean+z*SD were considered outbreak signals. An outbreak period began at the *nth* consecutive week when an outbreak signal was present, and ended when the same outbreak signal had been absent for *n* consecutive weeks.

The Shewhart Method

Logistic regression was performed on the alarm indicators and outbreak indicators within the historic period to determine associations between alarm and outbreak (2007/9 – 2011), known as the outbreak probability (Appendix 10). Specifically, the association between the value of the predictor variable in week 0 and an outbreak (yes=1, no=0) in the midpoint of the coming lag period (i.e. the mid-point of the lag period of 2-12 weeks is week 7) was determined using logistic regression. This was done per calendar week to obtain seasonality-adjusted estimates. Alarm data from all districts per country were used in the analysis assuming the same relationship existed between the alarm indicator and outbreak period across the country. Outbreak definitions were district specific. The resulting outbreak probability was compared with the weekly alarm threshold throughout the period of analysis (2012-13) to form alarm signals/ periods. The weekly alarm threshold was systematically tested between values of 0.6 – 2.0 to find a balanced environment within which alarms periods were formed *i.e.* when the outbreak probability was greater than the alarm threshold. Thereafter, defined alarm periods were used to detect defined outbreak periods during the period of analysis, and sensitivities/ positive predictive values (PPV) were used to evaluate performance. Alarm indicators, signals and periods were defined as follows:

Weekly Alarm indicator

1) Relative change in mean age of dengue incident cases (used a smoothed (average at week X - smoothed value week X-1)/smoothed value week X-1) due to noisy, low frequency data)
2) Number of probable cases (probable cases per 1,000 population)
3) Mean weekly outdoor temperature (weekly mean of daily means)
4) Total weekly rainfall
5) Mean weekly outdoor relative humidity (weekly mean of daily means)

Alarm signal: an alarm indicator with an outbreak probability above the alarm threshold occurring within the lag period.

Alarm period: a minimum of *n* alarm signals *i.e.* 1 per week for 3 weeks, which need not be consecutive, within the lag period. It is then immediately possible to form another alarm period, providing that alarm period does not coincide temporally with the outbreak period.

Lag Period

The lag period was used to determine the plausible time relation between an alarm and outbreak (time between a positive alarm signal and start of an outbreak) *i.e.* the timeframe before an outbreak within which a change in alarm variable could be related to subsequent dengue cases (Kroeger et al. 2014). This period ranged from 1 – 16 weeks before the outbreak depending upon the indicator selected e.g. mean humidity = 2-12 week period before the outbreak. Lag periods for each alarm signal were defined as follows:

- Temperature: 1 – 12 weeks before the outbreak
- Rainfall: 3 – 12 weeks before the outbreak

- Humidity: 2 – 12 weeks before the outbreak
- Mean Age: 4 – 16 weeks before the outbreak
- Breteau Index: 1 – 8 weeks before the outbreak
- House Index: 1 – 8 weeks before the outbreak
- Ovitrap Index: 2 – 8 weeks before the outbreak
- Probable Cases: 1 - 4 weeks before the outbreak (altered to 1-12 weeks due to few alarm periods)

Lag Time

Lag time was calculated as the period of time from the last alarm signal (*i.e.* the third week when the alarm was recorded positive/ last week of the alarm period) to the first outbreak signal in the outbreak period.

Sensitivity

The sensitivity was calculated as the number of outbreak periods detected by the alarm periods divided by the total number of outbreak periods. We are aware that the term 'sensitivity' is normally not used in statistical surveillance where repeated decisions are taken, but it is used in this paper to relate to traditional evaluations in fixed data sets.

Positive Predictive Value (PPV)

The PPV was calculated as the number of correct alarm periods, divided by the total number of defined alarm periods.

Specificity and negative predictive value were not used, as these required predicting negative events, which was not an aim of the model *i.e.* the model was not built to predict the absence of alarms and outbreaks.

Data Analysis

Analyses were run in duplicate, independently by two of the authors (LRB and MP) to limit systematic error. The Endemic Channel and Shewhart Method were programmed in Stata 13.1. In each case, an alarm (independent variable) and outbreak (dependent variable) were defined as the points at which the given variable recorded a higher absolute (or relative change in) value within the 2 years of analysis when compared against the historic ≥3-year probability or historic mean+z*SD respectively.

RESULTS

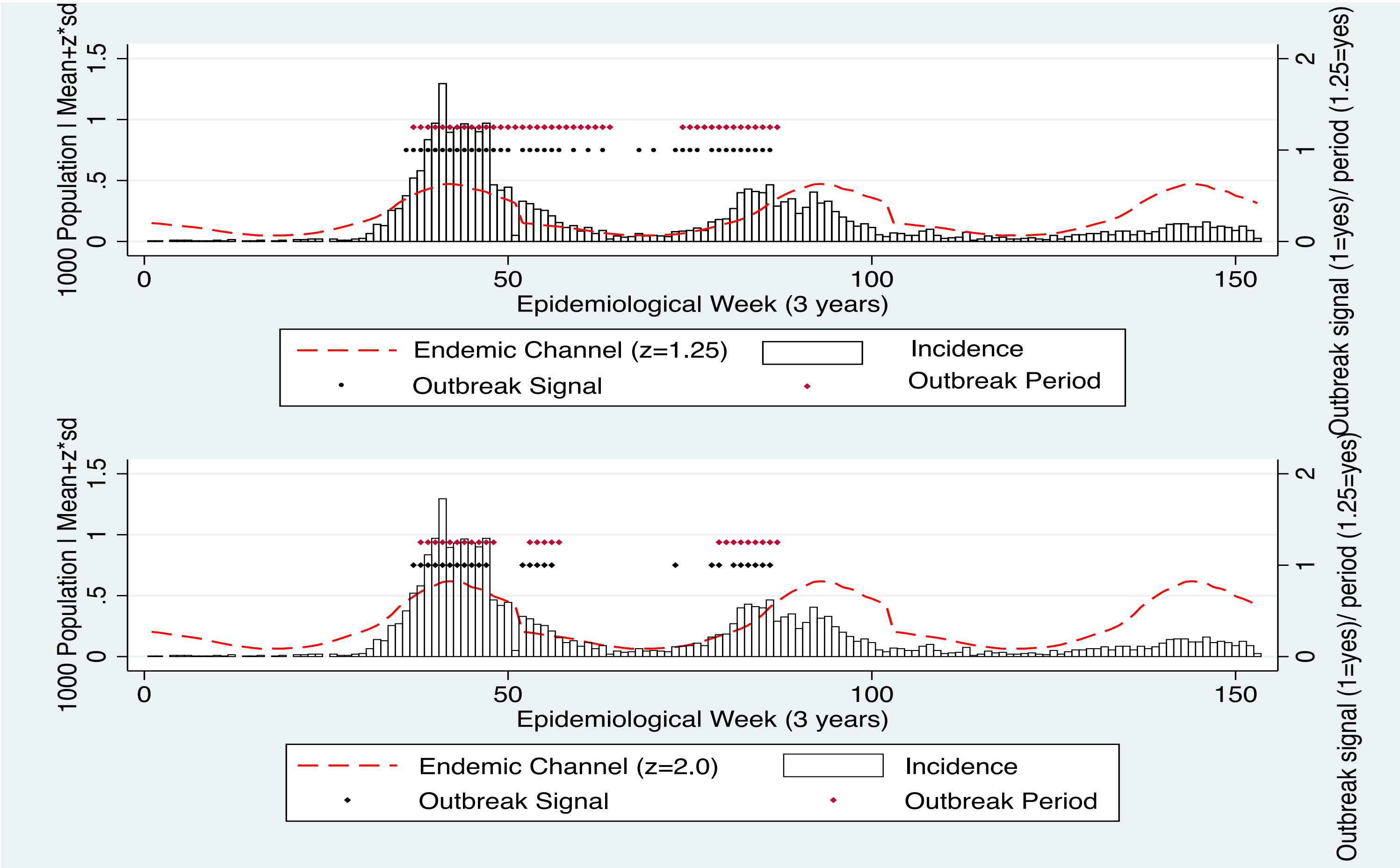
Development of the outbreak model using test data

As a starting point, multiple runs with test data were conducted to analyse the reliability of the model and consistency of the approach. The model was designed to a) define outbreak signals and form outbreak periods (Figure 5.1); b) adapt to changes in the z value by redefining the presence/ absence of outbreak signals/ periods; c) define alarm signals and form alarm periods based on the presence/ absence of outbreak signals/ periods; d) adapt to changes in the alarm threshold/ outbreak probability by redefining the presence/ absence of alarm signals/ periods; e) detect outbreak periods using alarm periods. In addition, false alarm periods were also measured. Intra-outbreak alarms were disregarded due to the way that these alarms would be considered prospectively *i.e.* in real time, alarms that occur within the outbreak would be considered only in the context of the continuing outbreak, and not future outbreaks, because any intervention measures implemented in response to the on-going outbreak would reduce the possibility that intra-outbreak alarms could be associated with future outbreaks (assuming that such interventions were effective in reducing dengue transmission to within the Endemic Channel).

Alarm and Outbreak Thresholds

Altering the z-value was the only method used to change thresholds and generate outbreak signals. As z was increased, fewer outbreak signals were generated (Figure 5.2). At a low z-value, outbreak signals were generated by relatively low magnitude incidence, and were continually recorded for long durations (Figure 5.2). Thereafter, as the z-value increased, lower magnitude incidence did not form outbreak signals, and the number of outbreak signals became less frequent.

Figure 5.2. Modelling with test data using two z values (1.25, 2.0) to form thresholds known as the Endemic Channel. Outbreak periods begun by *n* = 2 consecutive outbreak signals and ended when *n* = 2 consecutive outbreak signals; top: z-value = 1.25; bottom: z-value = 2.0.



In a similarly systematic approach, the outbreak probability, defined as the relationship between historic dengue incidence and alarm indicators, was used to quantify alarm signals prior to outbreaks during the period of evaluation (2012-13) (Figures 5.3 and 5.4). As previously observed with z values and outbreak signals, alarm signal frequency also decreased as the alarm threshold was increased (Figures 5.3 and 5.4).

Figure 5.3. Modelling with test data to predict outbreak periods using alarm periods at alarm threshold = 0.12. Alarm periods (defined by n = 2 alarm signals) successfully detecting outbreak periods (defined by n = 2 outbreak signals); false alarms also highlighted; during outbreak alarms discounted; z-value = 1.25.

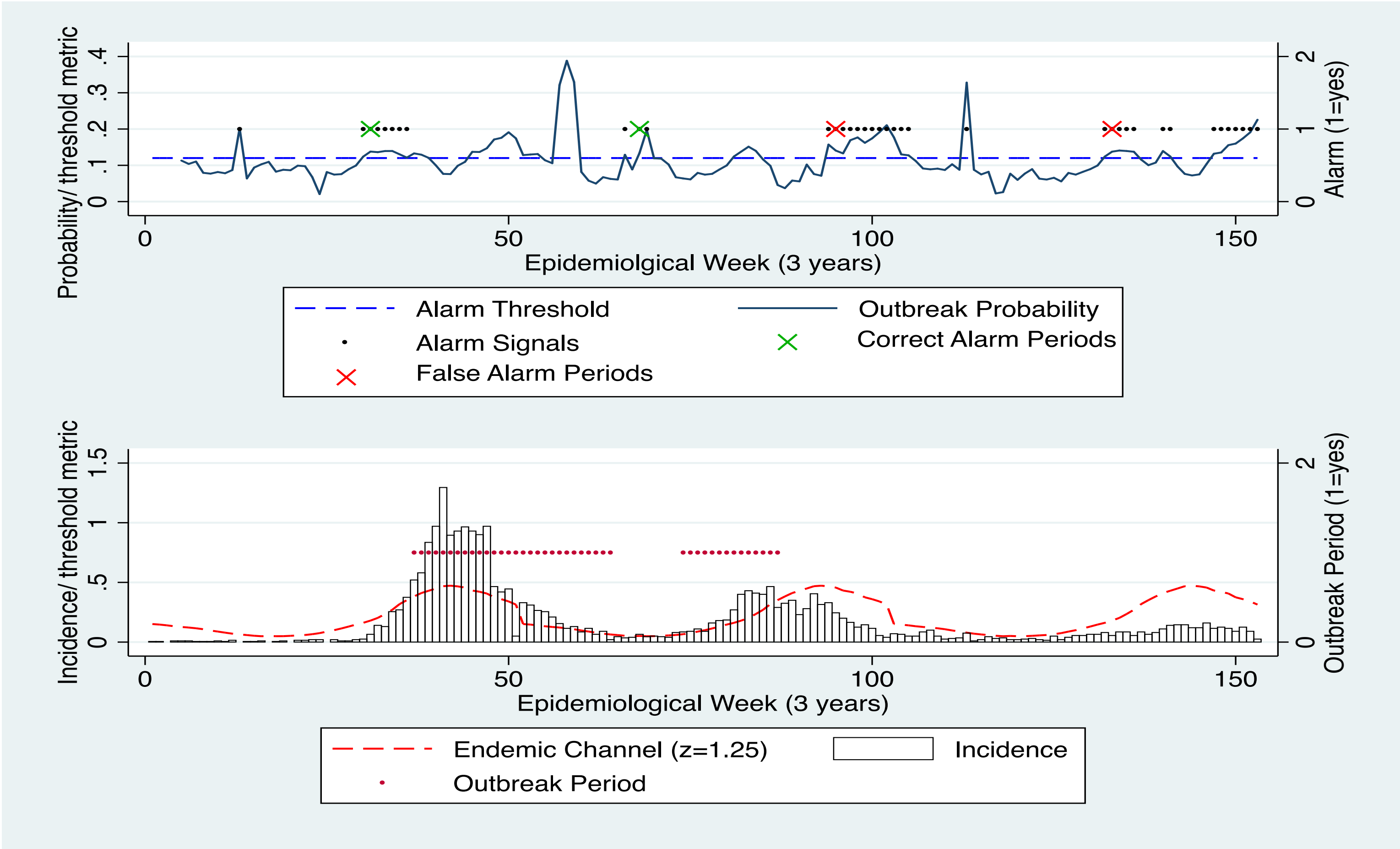
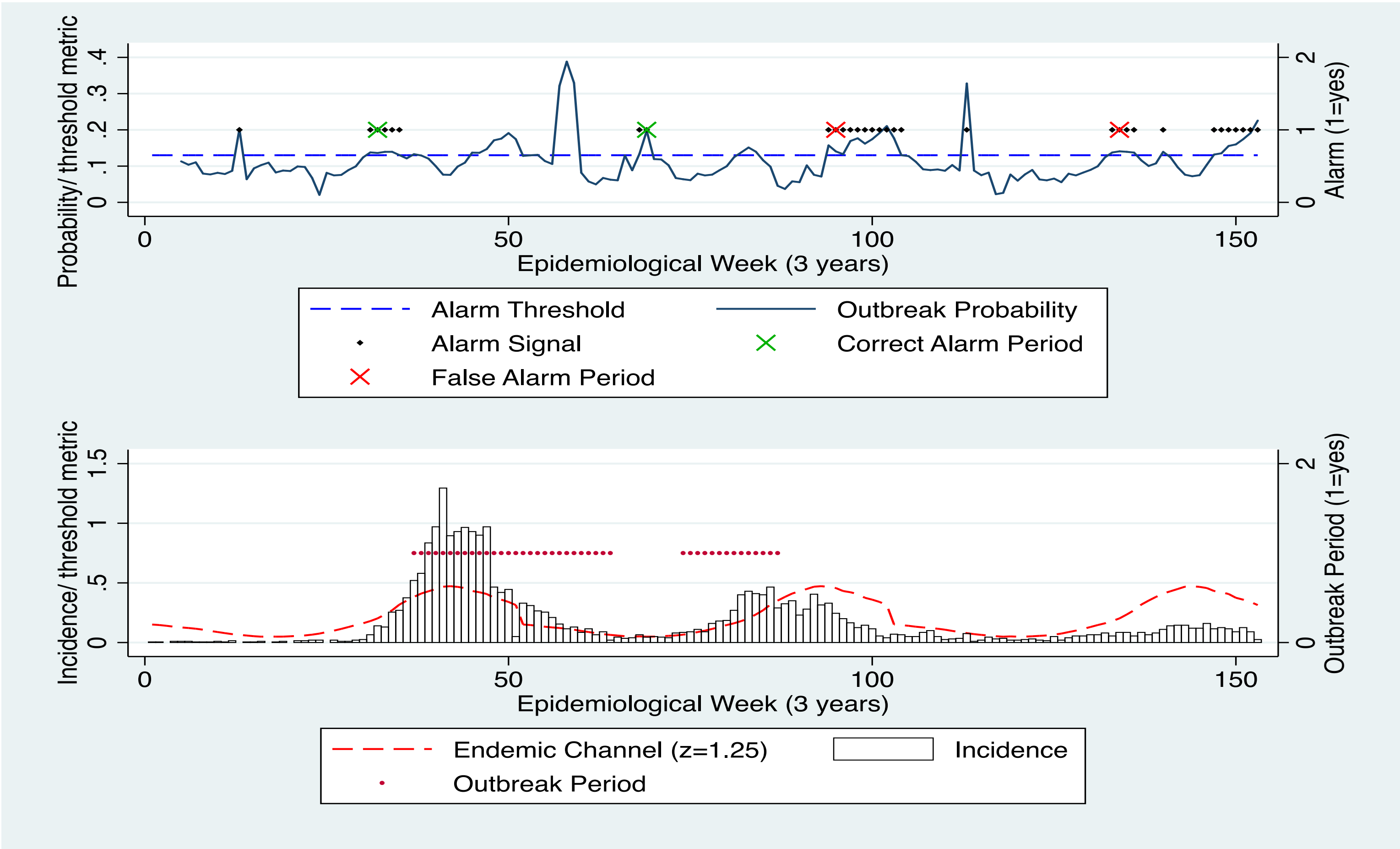


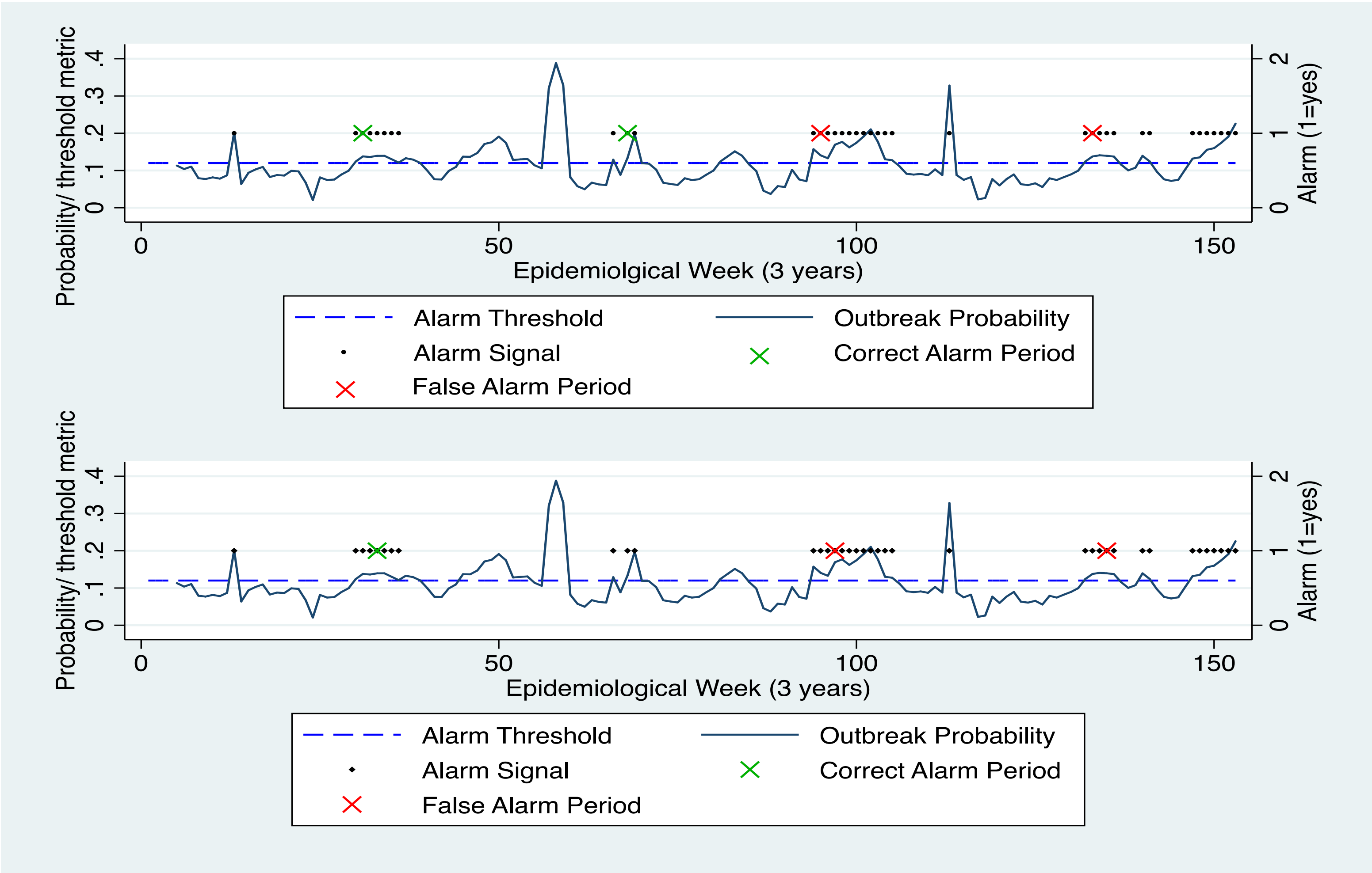
Figure 5.4. Modelling with test data to predict outbreak periods using alarm periods at alarm threshold = 0.13. Alarm periods (defined by *n* = 2 alarm signals) successfully detecting outbreak periods (defined by *n* = 2 outbreak signals); false alarms also highlighted; during outbreak alarms discounted; alarm threshold of 0.13 to detect outbreaks generated by z = 1.25.



Alarm and Outbreak Definitions

In an effort to reduce the influence of spurious/ anomalous data, multiple alarm signals and outbreak signals were used to form alarm and outbreak periods, (Figures 5.2 and 5.5). To ensure that detection times were reasonably short, an *n*-value of 2, 3 and 4 alarm/ outbreak signals was used to define both alarm and outbreak periods. Altering the number of signals required to form an alarm/ outbreak period increased or decreased the frequency of alarm/ outbreak periods (Figure 5.5), and also affected the temporal relationship between alarm and outbreak periods by altering the week that alarm/ outbreak periods were observed (Figure 5.5). In prospective terms, as *n* increases, outbreak detection times are delayed.

Figure 5.5. Modelling with test data to predict outbreak periods using alarm periods, where variation in *n* creating alarm periods is observed. Alarm threshold = 0.12, z = 1.25; top, alarm periods defined by *n* = 2 alarms signals; bottom, alarm periods defined by *n* = 4 alarm signals.



Notably, increasing the z-value could increase the frequency of outbreak periods. While this may be counterintuitive, consider Figure 5.2, where z=2.0 captured only the peaks of incidence. In this figure, using a higher z-value split one outbreak period into two, thereby increasing the number of outbreak periods available for detection, albeit shortening the frequency of outbreak weeks (Figure 5.2). As the z-value was increased further, outbreak signals became fewer, which decreased the length and ultimately the frequency of outbreak periods.

Mean Lag Time

As an unexpected consequence of the interdependency of lag time on the alarm threshold, alarm periods were often formed at a progressively closer temporal proximity to the outbreak as the alarm threshold increased (Figures 5.3 and 5.4). In other words, gradually increasing alarm thresholds captured only the highest peaks in outbreak probability, thus forming alarm signals temporally later and so reducing the time between the beginning of an alarm period and an outbreak period. Consequently, the mean lag time gradually decreased as alarm thresholds increased (Table 5.1). In addition, an increase or decrease in the number of alarm signals used to create alarm periods also shifted the temporal relationship between alarm periods and outbreak periods to the right *i.e.* delayed detection times (Figure 5.5). Given this interdependency, it was clear that model parameterisation had a greater impact on the temporal associations between alarm indicators and outbreaks, masking true associations. Hence, no further results were generated for this outcome.

Table 5.1. Test data results showing model performance metrics. Row 5 of each z-value shows mean lag times (weeks) decreasing as the outbreak probability increases in spite of a change in z level.

Output	**Z-value**	**Probability**						
		0.08	**0.1**	**0.12**	**0.14**	**0.16**	**0.18**	**0.2**
Mean number of outbreaks	**1.25**	4.33	4.33	4.33	4.33	4.33	4.33	4.33
Mean number of alarm periods		9.67	9.67	9.67	9.67	9.67	10.00	10.00
Proportion outbreaks detected (sensitivity)		1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proportion correct alarm periods out of all alarm periods (~PPV)		0.42	0.42	0.42	0.42	0.42	0.40	0.40
Mean lag time for alarm		9.58	9.58	9.58	9.58	9.50	9.30	9.23
Mean number of outbreaks	**1.5**	4.33	4.33	4.33	4.33	4.33	4.33	4.33
Mean number of alarm periods		9.67	9.67	9.67	9.67	9.67	10.00	10.00
Proportion outbreaks detected (sensitivity)		1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proportion correct alarm periods out of all alarm periods (~PPV)		0.42	0.42	0.42	0.42	0.42	0.40	0.40
Mean lag time for alarm		9.58	9.58	9.58	9.58	9.50	9.30	9.23

Model Performance Evaluation

After demonstrating the functionality of the model (Figures 5.1 – 5.5), outcome metrics were required to quantitatively evaluate the detection system and determine the applicability of the model to different country contexts. In this regard, the use of alarm periods to detect outbreak periods provided an opportunity to evaluate the detection performance of the model. The performance metrics used were sensitivity and positive predictive value, as defined above. These outcome metrics were used to evaluate the applicability and predictive performance of the model, initially done using 3 validated retrospective datasets (Malaysia, Mexico and Brazil), before expanding the analysis to Viet Nam and Dominican Republic datasets.

Firstly, z-values and outbreak probabilities were determined. It was important to evaluate these thresholds to ensure that sufficient outbreak periods were created to enable detection by alarm periods. A systematic approach ensured that all z and alarm threshold values were tested incrementally. Alarm thresholds were evaluated against a constant z of 1.25 (Figure 5.6), while z values were tested in a similar fashion against a constant outbreak probability of 0.12 (Figure 5.7). Results indicated that, despite altering the alarm and outcome variables, a z-value of between 1.0 - 1.3, and an outbreak probability of between 0.06 and 0.12, yielded the best performance metrics (highest sensitivities/ PPVs) (Figures 5.6 and 5.7). Higher coefficients of either outbreak probabilities or z-values resulted in marked decreases in sensitivity, and to some extent, in PPV (Figures 5.6 and 5.7). Hence, a coefficient of z = 1.25/ outbreak probability = 0.12 were used to define the outbreak/ alarm thresholds and thus to evaluate all remaining alarm and outcome variables within each dataset (Table 5.2).

Figure 5.6. Performance testing of the outbreak probability using 3 country datasets where the z-value = 1.25. Alarm/ outbreak periods define by *n*=3; Brazil: Alarm indicator = Probable Cases; Outbreak indicator = Hospitalised Cases; Mexico: Alarm indicator = mean temperature; Outbreak indicator = Hospitalised Cases; Malaysia: Alarm indicator = Mean age; Outbreak indicator = Hospitalised Cases.

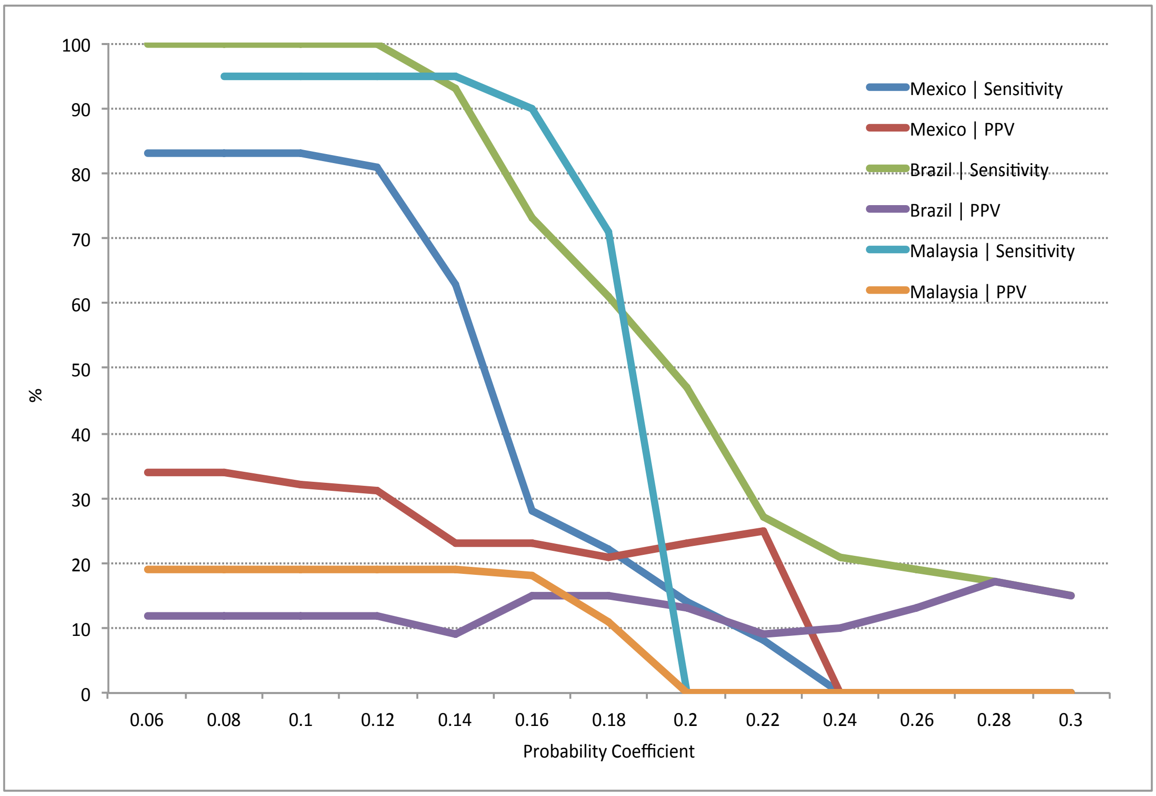


Figure 5.7. Performance testing of the z-value using 3 country datasets where the outbreak probability = 0.12. Alarm/ outbreak periods defined by *n*=3; Mexico: Alarm indicator = Weekly mean temperature; Outbreak indicator = Hospitalised Cases; Brazil: Alarm indicator = Probable Cases; Outbreak indicator = Hospitalised Cases; Malaysia: Alarm indicator = Mean Age; Outbreak indicator = Hospitalised Cases.

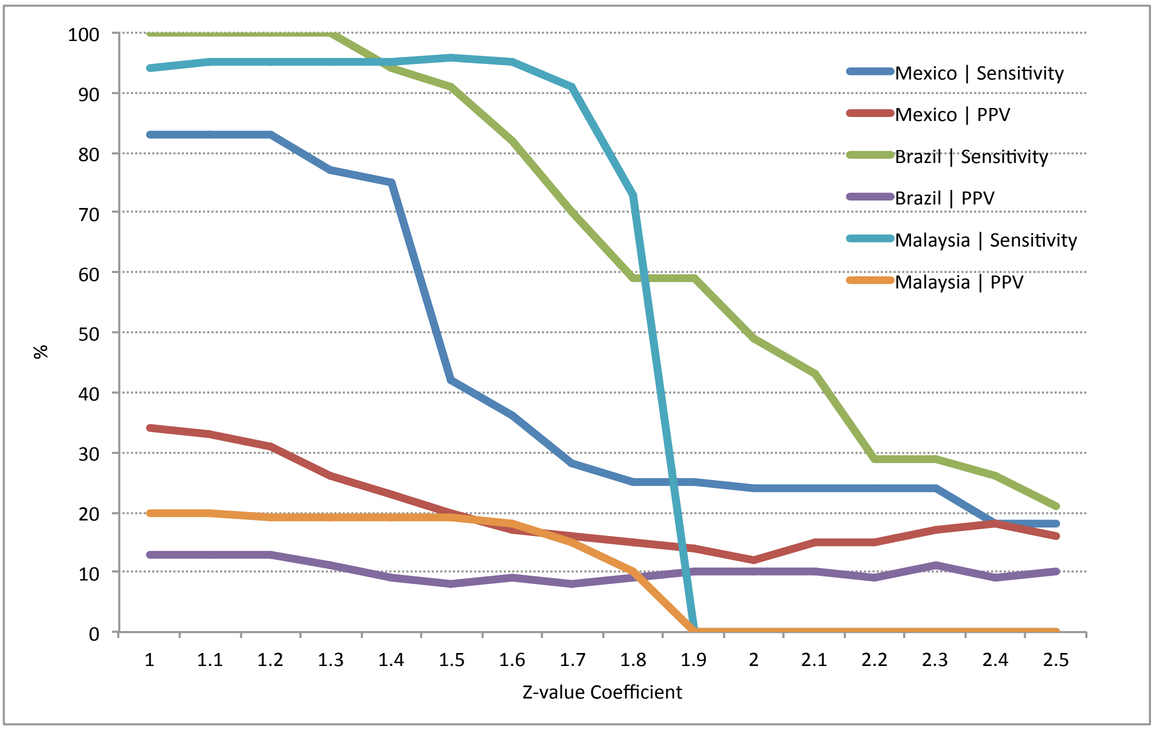


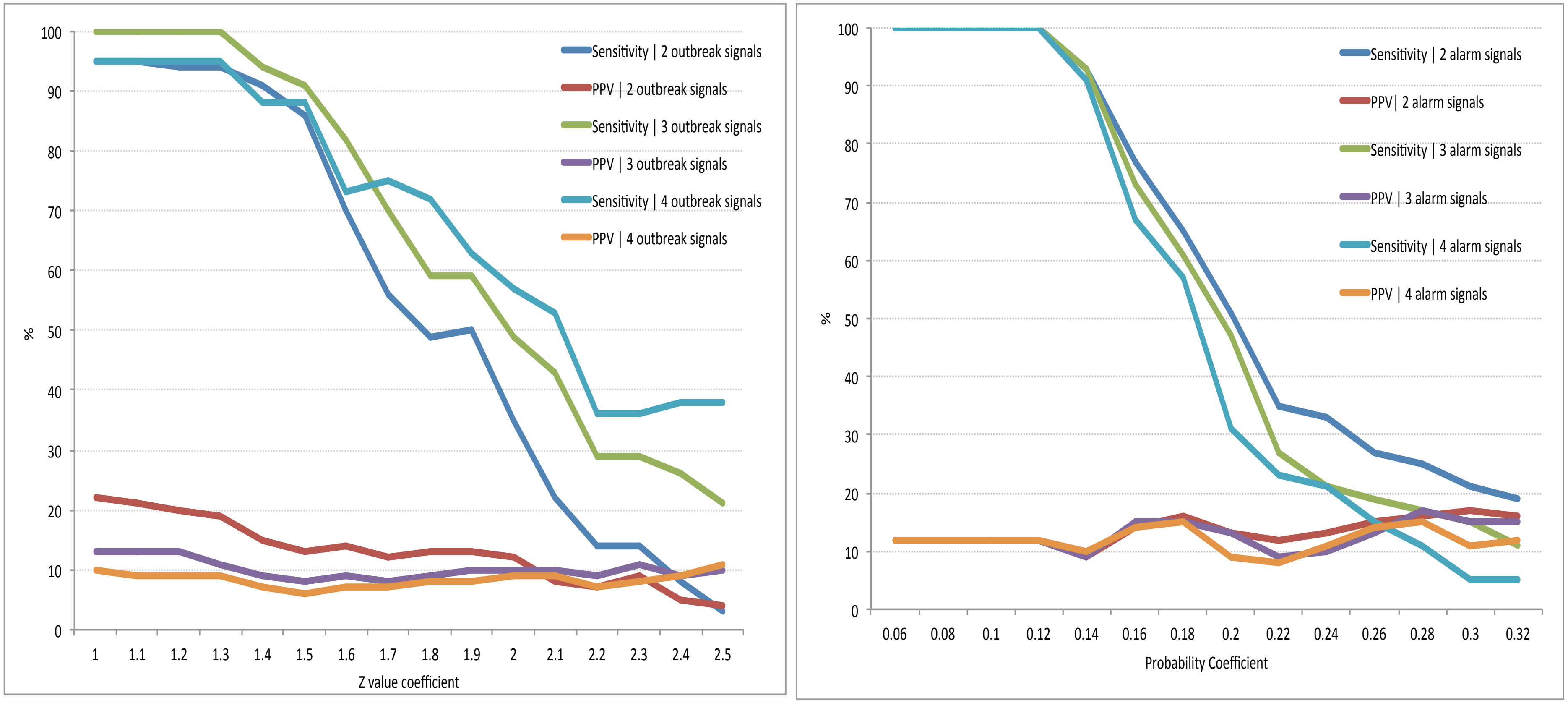
Table 5.2. Summary results table stratified by country. Most sensitive indicators stratified by country where z=1.25 and probability = 0.12

Country	Indicator	Outbreak indicator	Lag Period (weeks)	Sensitivity (%)	Positive Predictive Value (%)
Mexico	Mean Temperature	Hospitalised Cases	1-12	81	35
Mexico	Rainfall	Hospitalised Cases	3-12	63	27
Mexico	Mean Age	Hospitalised Cases	4-16	73	35
Mexico	Probable Cases	Hospitalised Cases	1-12	91	40
Brazil	Mean Temperature	Hospitalised Cases	1-12	84	10
Brazil	Probable Cases	Hospitalised Cases	1-12	100	12
Brazil	Rainfall	Hospitalised Cases	3-12	67	8
Brazil	Mean Humidity	Hospitalised Cases	2-12	88	13
Brazil	Mean Temperature	Probable Cases	1-12	52	17
Brazil	Mean Age	Hospitalised Cases	4-16	100	12
Malaysia	Mean Age	Probable Cases	4-16	99	19
Malaysia	Mean Temperature	Probable Cases	1-12	14	25
Malaysia	Mean Humidity	Probable Cases	2-12	10	21

Country	Indicator	Outbreak indicator	Lag Period (weeks)	Sensitivity (%)	Positive Predictive Value (%)
Dominican Republic	Rainfall	Hospitalised Cases	3-12	18	41
Dominican Republic	Mean Temperature	Hospitalised Cases	1-12	23	43
Dominican Republic	Mean Humidity	Hospitalised Cases	2-12	6	42
Dominican Republic	Probable Cases	Hospitalised Cases	1-12	92	46
Dominican Republic	Mean Humidity	Probable Cases	2-12	5	39
Dominican Republic	Mean Temperature	Probable Cases	1-12	23	43
Dominican Republic	Rainfall	Probable Cases	3-12	16	38
Vietnam	Mean Age	Probable Cases	4-16	52	11
Vietnam	Probable Cases	Hospitalised Cases	1-12	87	12

To evaluate the impact of detection times, alarm and outbreak periods were defined using an *n* of 2, 3 or 4. At an alarm threshold of 0.12 where the z was systematically increased from 1.0 – 2.5 by increments of 0.1, the analyses showed that using 2 or 3 outbreak signals to form the outbreak period generated highest model performance metrics, as can be seen in Figure 5.8. Similarly, where z=1.25, alarm period definitions were tested using an *n* of 2, 3 or 4 alarm signals. During this particular evaluation, the variation in performance metrics was not as significant as observed when defining outbreak periods. Nonetheless, the highest model performance was observed when using 2 and 3 alarm signals (Figure 5.8).

Figure 5.8. Performance testing of the *n* alarm/ outbreak signals creating alarm and outbreak periods using the Brazil dataset. Alarm indicator = probable cases; Outbreak indicator = hospitalised cases; left: outbreak periods defined where *n* = 2, 3 and 4 weekly outbreak signals; right: alarm periods defined where *n* = 2, 3 and 4 weekly alarm signals.



At this point, univariate analyses were conducted using combinations of n = 2 and 3 at a z = 1.25 and outbreak probability of 0.12, to highlight any small differences there were in the performance metrics between both approaches.

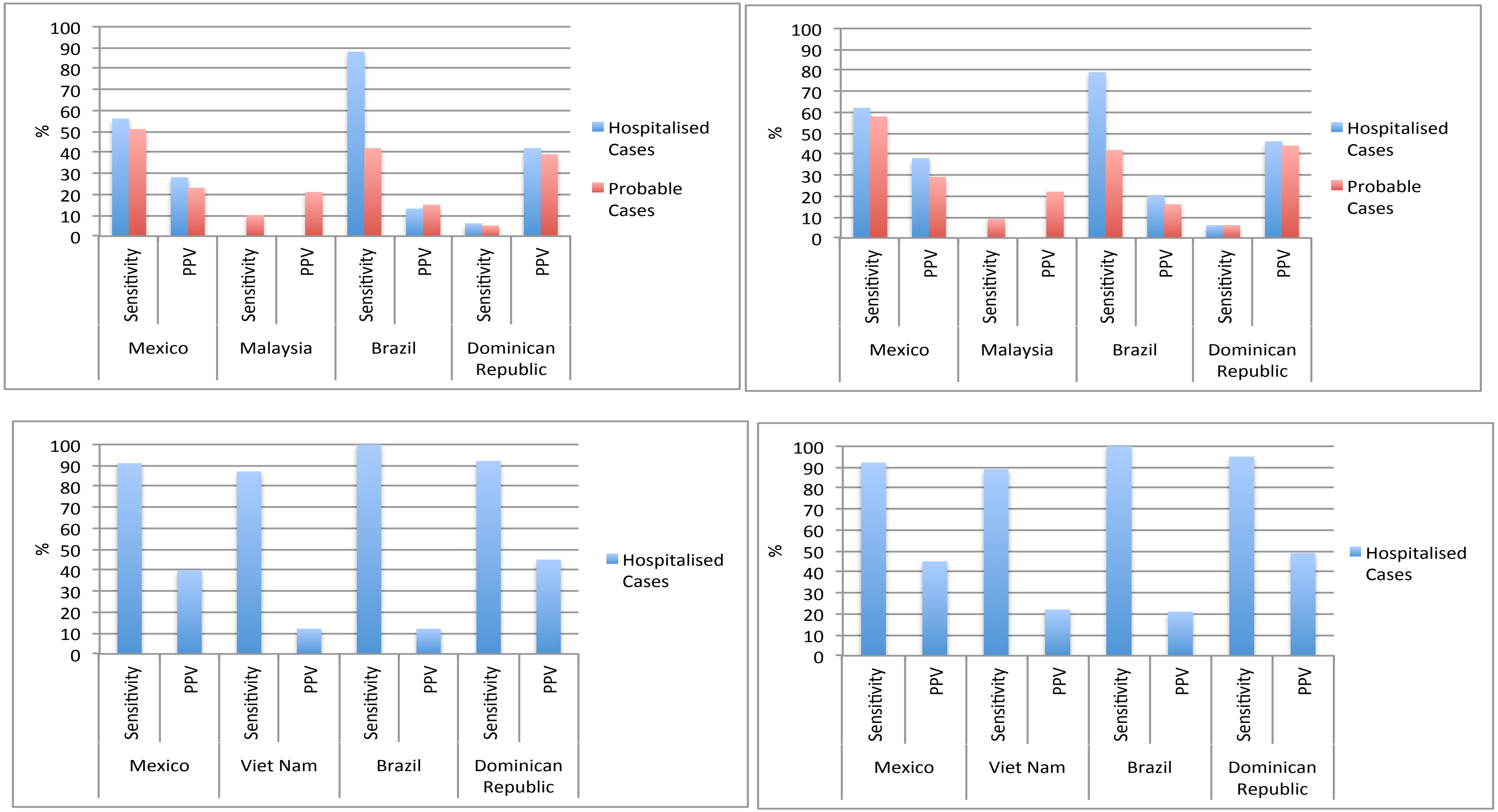
Univariate Analyses

Epidemiological Variables

Alarm Indicator: Probable cases - Outbreak Indicator: Hospitalised cases

Use of probable cases was highly sensitive for predicting outbreak periods, where hospitalised cases was used as the outbreak indicator, with all sensitivities across each country recorded as ≥89% (Figure 5.9). Sensitivity decreased to a minimum of 87% when n = 3 for alarm/ outbreak periods (Figure 5.9). In both Mexico and Dominican Republic, PPV values were 45% and 49% respectively, while among Brazil and Viet Nam datasets, values were low at 22% and 21% respectively.

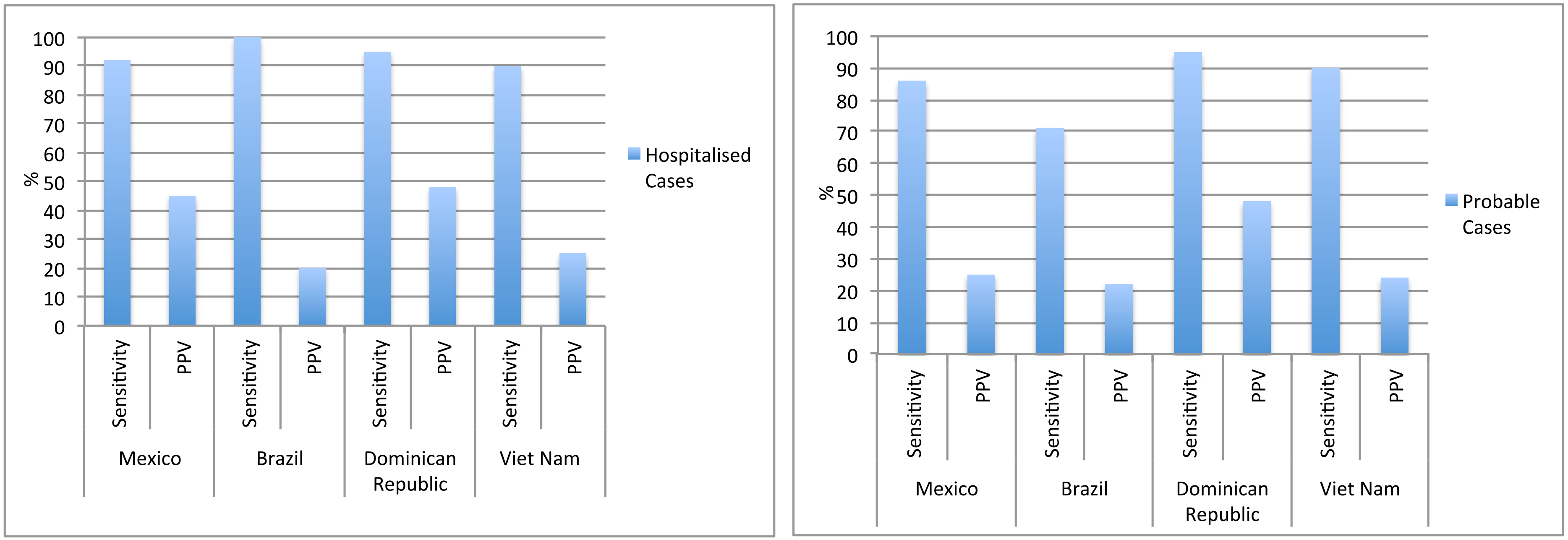
Figure 5.9. Univariate analyses showing sensitivities and PPVs using multiple country datasets. Outbreak probability = 0.12, z-value = 1.25. Top: Alarm indicator = Humidity. Bottom: Alarm indicator = Probable Cases. Left: alarm periods defined by n = 3; outbreak periods defined by n = 3; Right: alarm periods defined by n = 2; outbreak periods defined by n = 2.



Alarm Indicator: Hospitalised cases - Outbreak indicator: Hospitalised cases

Performance metrics were high in terms of sensitivity, with all country datasets recording ≥90% where n = 2, but still false alarm periods were generated roughly 50% of the time for Mexico and Dominican Republic, 45% and 48% respectively (Figure 5.10), while PPV values were considerably lower in Brazil and Viet Nam, at 20% and 25% respectively (Figure 5.10). Both Mexico and Dominican Republic datasets recorded the highest, combined model performance (Figure 5.10).

Figure 5.10. Univariate analyses showing sensitivities and PPVs using multiple country datasets. Outcome indicators were used as alarm indicators where outbreak probability = 0.12 and z=1.25; 2 alarm/ outbreak signals to define alarm/ outbreak periods; left: alarm indicator = hospitalised cases; outbreak indicator = hospitalised cases; right: alarm indicator = probable cases; outbreak indicator = probable cases.



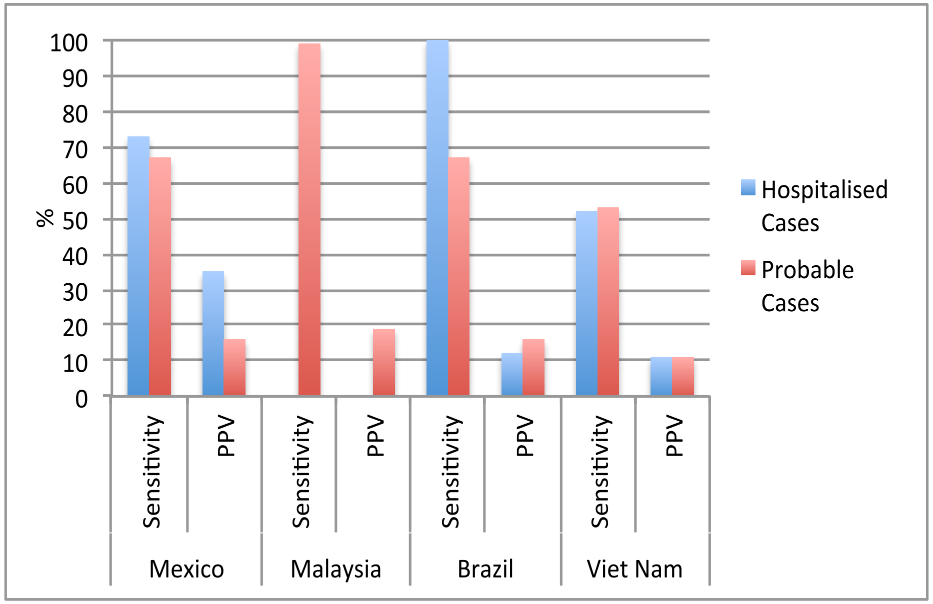
Alarm Indicator: Probable cases - Outbreak indicator: Probable cases

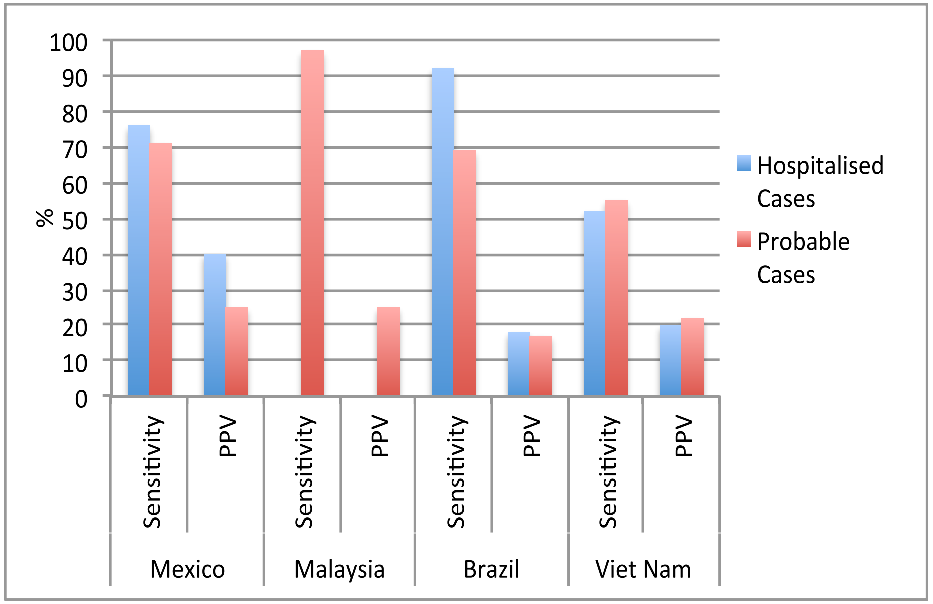
Unfortunately, paucity of data during week 23 caused the model to terminate when analysing the Malaysia dataset, although analyses were possible with the remaining country datasets. While performance metrics using probable cases were high (sensitivities no lower than 71%), PPV values dropped from those observed when the outbreak indicator was hospitalised cases. The Mexico dataset precipitated reductions from 45% to 25%, and were consistently low for all remaining country datasets, excepting Dominican Republic, which performed at a consistent level between both outbreak indicators (Figure 5.10). In light of this, only hospitalised cases were taken forward into multivariate analyses, as they were clearly the stronger incidence-based outbreak indicator.

Alarm Indicator: Mean age of hospitalised cases - Outbreak indicator: Hospitalised Cases

A change in mean age (increase or decrease) yielded moderate sensitivities across country datasets at a lag of 4-16 weeks. Where n = 2 for alarm and outbreak periods, performance metrics were generally higher across all countries (Figure 5.11), but this difference was modest and the variation between countries was high (Figure 5.11). The highest PPV was not greater than 40% (Mexico) (Figure 5.11), and mean age could not be analysed in Dominican Republic due to a paucity of data.

Figure 5.11. Univariate analyses showing sensitivities and PPVs for multiple country datasets. Outbreak probability = 0.12, z-value = 1.25. Alarm indicator: Mean Age. Outbreak indicator: probable cases; hospitalised cases. Left: alarm periods defined by *n* =3; outbreak periods defined by *n* = 3; Right: alarm periods defined by *n* = 2. Outbreak periods defined by *n* = 2.





Alarm Indicator: Mean age of probable cases - Outbreak indicator: probable cases.

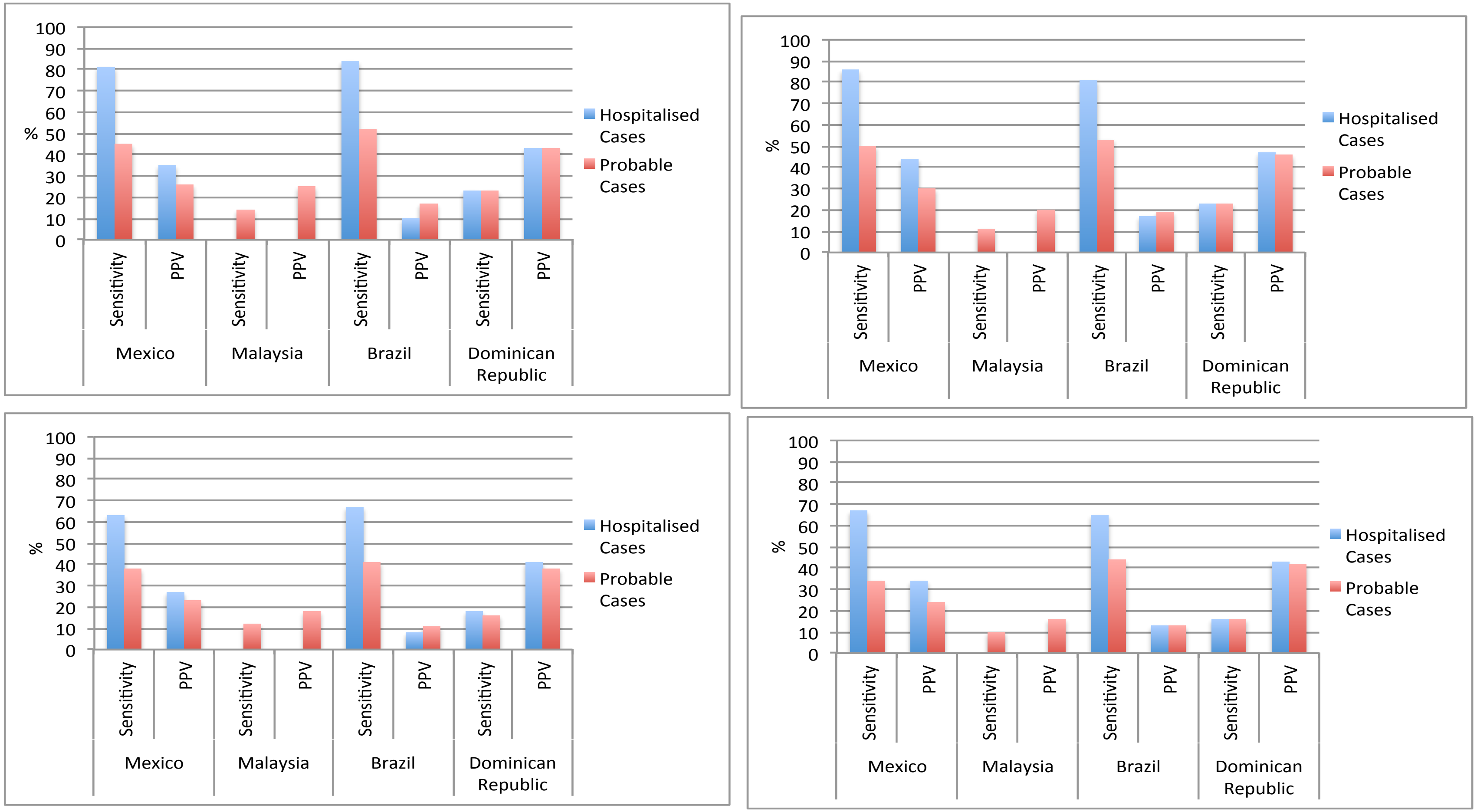
Where the outbreak indicator was probable cases, all performance metrics, were notably lower than when hospitalised cases was the outbreak indicator (Figure 5.11). Accordingly, this combination was subsequently dropped from multivariate analyses.

Meteorological Variables

Alarm Indicator: Mean temperature. Outbreak indicator: hospitalised cases

Of the meteorological variables, mean temperature recorded higher performance metrics than either rainfall or humidity (Figure 5.12). In Mexico and Dominican Republic, PPVs were greater than 40% where n = 2 or 3, while PPVs in Brazil and Malaysia were approximately 20% (Figure 5.12). Modest differences in performance were detected between n-values of 2 and 3 for alarm/ outbreak periods, with the former recording marginally improved results (Figure 5.12). Sensitivities were highest in Mexico and Brazil (≥80%), while Mexico recorded the best, combined performance, with sensitivity of 86% and PPV of 44% (Figure 5.12).

Figure 5.12. Univariate analyses showing sensitivities and PPVs using multiple country datasets. Top: Weekly mean temperature. Bottom: Weekly total rainfall. Outbreak probability = 0.12, z-value = 1.25 Left: alarm periods define by *n* = 3; outbreak periods defined by *n* = 3; Right: alarm periods defined by *n* =2; outbreak periods defined by *n* = 2.



Alarm Indicator: Weekly mean temperature. Outbreak indicator: Probable cases

Across those datasets where the outbreak indicator could have been either hospitalised or probable cases, probable cases was generally a poorer variable, except for Dominican Republic, where model performances were extremely similar. Again, best performance was observed with the Mexico dataset (sensitivity 50%, PPV 30%) (Figure 5.12). Where *n* = 2, this generally improved performance, although the difference was marginal (Figure 5.12). Sensitivities were substantially higher in two country datasets (Mexico 50%, Brazil 53%) (Figure 5.12).

Alarm Indicator: Weekly total rainfall. Outbreak indicator: hospitalised cases

Model performance metrics for rainfall were modest, with sensitivities achieving 65% and 67% in Brazil and Mexico, however PPV values were only 34% and 14% respectively (Figure 5.12). This was marginally different where *n* = 3, resulting in sensitivities of 63% and 67% (Figure 5.12), though PPVs were lower in both cases. The remaining country datasets recorded low to moderate performance for both metrics (<50% (Figure 5.12)).

Alarm Indicator: Weekly total rainfall. Outbreak indicator: probable cases

In all countries, performance metrics were lower when using probable cases as the outbreak indicator. Neither sensitivities nor PPV values were above 50% for any country dataset, nor was this altered by changing the *n* required for alarm/ outbreak periods (Figure 5.12).

Alarm Indicator: Weekly mean humidity. Outbreak indicator: hospitalised cases

Weekly mean humidity demonstrated predictive potential as an alarm indicator between countries, where *n* = 3 in Brazil, resulting in a performance sensitivity of 88%, however PPV was much lower at 13% (Figure 5.9). Otherwise, all other country datasets recorded higher performance where *n* = 2 for alarm/ outbreak periods, but even so, performance was moderate at best (Figure 5.9).

Alarm Indicator: Weekly mean humidity. Outbreak indicator: probable cases

Following an emerging theme, here also it was clear that a change in outbreak indicator reduced the performance of the model, except with the Dominican Republic data. On one occasion, sensitivity was much lower (Brazil: sensitivity 42% vs. 88% where the outcome was hospitalised cases), but other performance metrics were only marginally worse.

NB: No meteorological correlations could be analysed for Vietnam as data were not available.

Entomological Variables

No results were generated for any country dataset as entomological datasets were deemed too inconsistent and/ or included highly variable data collection methodologies.

Multivariate Analyses

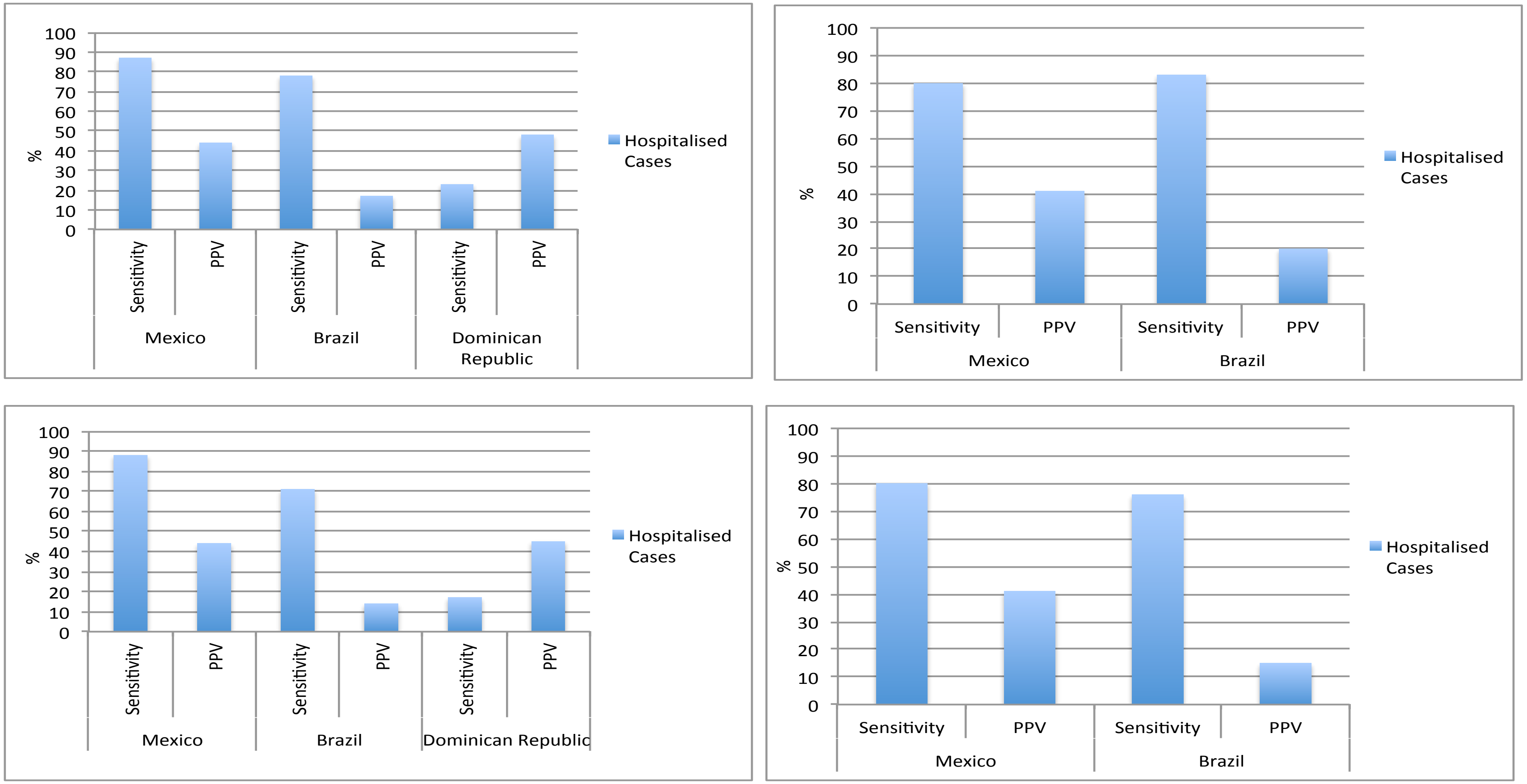
Meteorological Variables

Given the natural existence of linear relationships between meteorological variables, multivariate analyses for these variables would not identify singly predictive variables and was not conducted. However, meteorological and epidemiological variables are unlikely to be connected in this way, thus multivariate analyses were conducted using the strongest singly predictive meteorological and epidemiological alarm indicators, where the outbreak indicator was consistently hospitalised cases. The lag period was altered to include the entire range of the combined variables in question *e.g.* lag period was defined as 1-16 weeks when combining both mean temperature (lag period of 1-12 weeks) and mean age (lag period of 4-16 weeks). All model testing took place using a z-value of 1.25 and outbreak probability of 0.12, where n = 2 to form alarm/ outbreak periods.

Alarm Indicator: Weekly mean temperature; probable cases. Outbreak indicator: hospitalised cases

Of the three datasets that could be analysed with the above indicators, Mexico demonstrated the highest performance metrics, with sensitivity of 87% and PPV value of 44% (Figure 5.13). Neither Brazil nor Dominican Republic data demonstrated meaningful performance metrics (Figure 5.13).

Figure 5.13. Multivariate analyses showing sensitivities and PPVs using multiple country datasets. Outbreak probability = 0.12, z-value = 1.25. *n* = 2 for alarm/ outbreak periods; top left: alarm indicators = weekly mean temperature, probable cases, 1-12 weeks; top right: alarm indicators = weekly mean temperature, mean age, 1-16 weeks; bottom left: alarm indicators = weekly total rainfall, probable cases, 1-12 weeks; bottom right: weekly alarm indicators = rainfall, mean age, 1-16 weeks; outbreak indicator = hospitalised cases.



Alarm Indicator: Weekly mean temperature; mean age. Outbreak indicator: hospitalised cases

Only two country datasets could be used in this analysis due to paucity of data. Sensitivities were relatively high ≥80%, while PPV values were 41% and 20% for Mexico and Brazil respectively (Figure 5.13).

Alarm Indicator: Weekly total rainfall; probable cases. Outbreak indicator: hospitalised cases

Mexico data revealed relatively high performance metrics when compared with two other country datasets (Brazil, Dominican Republic), with sensitivity of 88% and PPV value of 44% (Figure 5.13). Performance metrics using the latter country datasets was inconsistent, either with high sensitivities and low PPVs (Brazil), or vice versa (Dominican Republic) (Figure 5.13).

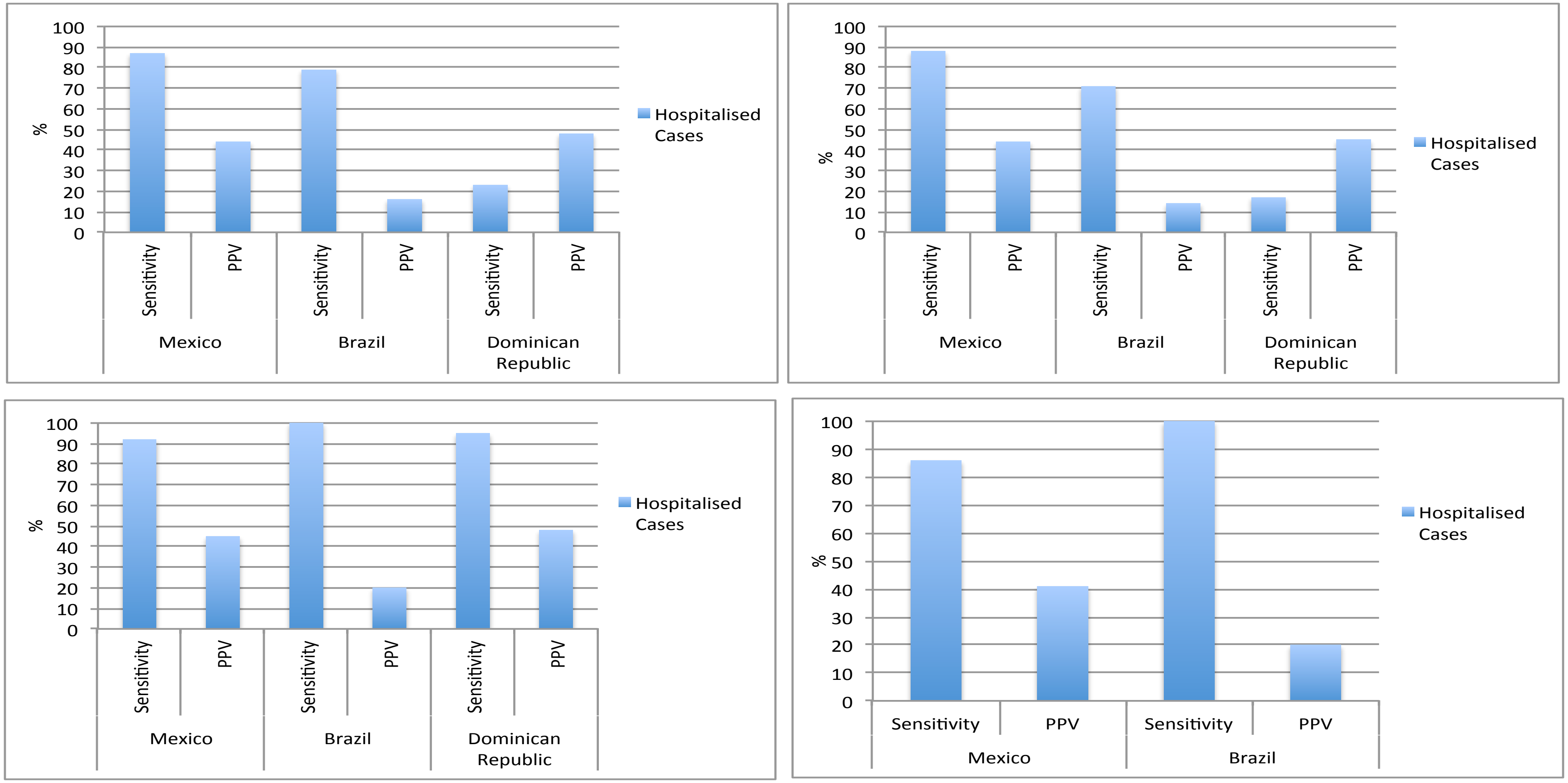
Alarm Indicator: Weekly total rainfall; mean age. Outbreak indicator: hospitalised cases

Continuing a trend observed throughout the multivariate analyses, PPV values using the Mexico dataset were substantially higher than with the Brazil dataset, 41% vs. 15% respectively, while sensitivities were relatively similar (80%, 83% respectively) (Figure 5.13).

Alarm Indicator: Weekly mean temperature; hospitalised cases. Outbreak indicator: hospitalised cases

Sensitivities were high in Mexico and Brazil, 79% and 87% respectively, however PPV values generated with the Brazil dataset were low at 16%, while in Mexico values were moderate at 44%. Dominican Republic data produced moderate PPV values at 48%, but sensitivity was low at 23% (Figure 5.14).

Figure 5.14. Multivariate analyses showing sensitivities and PPVs using multiple country datasets. Outbreak probability = 0.12; z-value = 1.25; lag period = 1-12 weeks; *n* = 2 for alarm/ outbreak periods; outbreak indicator: hospitalised cases. top left: alarm indicators = mean temperature, hospitalised cases; top right: alarm indicators = rainfall, hospitalised cases; bottom left: alarm indicators = probable cases, hospitalised cases; bottom right, alarm indicators = mean age, hospitalised cases.



__Alarm Indicator: Weekly total rainfall; hospitalised cases. Outbreak indicator: hospitalised cases__

Sensitivities were high in both Mexico and Brazil, as observed elsewhere, at 88% and 71% respectively, while PPV values were 44% and 14% respectively. Dominican Republic sensitivities were low, while PPV was moderate at 48% (Figure 5.14).

Epidemiological Variables

__Alarm Indicator: Probable cases; hospitalised cases. Outbreak indicator: hospitalised cases__

Here, Dominican Republic data performed particularly well, with performance metrics of sensitivity 95% and PPV value of 48%. Mexico data also recorded moderate - high performance metrics, with 92% and 45% sensitivity and PPV respectively. Although sensitivity was high with the Brazil dataset (100%), PPV was still relatively low (20%) (Figure 5.14).

__Alarm Indicator: Mean age; hospitalised cases. Outbreak indicator: hospitalised cases__

These indicators showed potential among the Mexico dataset, where sensitivity was 86% and PPV 41%, yet Brazil PPV values were low at 20%, detracting from the success of high sensitivities (100%) (Figure 5.14).

DISCUSSION

Univariate Analyses

Clearly, alarm indicators that provide advance warning of outbreak periods are the most valuable, in order to enact timely clinical preparations and vector control responses. From an operational perspective, it is equally important that these alarm indicators are not wrong too often *i.e.* high positive predictive value. Arguably the main driver behind this is confidence in the model – too many false alarms will lead to unnecessary interventions and wasted resources. In this study, the Shewhart method and Endemic Channel were used to identify alarm indicators with the potential to predict subsequent outbreak periods. A number of indicators were tested, ranging from epidemiological to meteorological, to evaluate primarily their predictive potential, and secondarily the most appropriate measure of dengue incidence to define outbreaks.

Despite inherent variability throughout the datasets, certain meteorological and epidemiological alarm indicators were potentially predictive across all countries, which is consistent with trends and evidence reported elsewhere (Phung et al. 2015; Hii et al. 2009; Hii et al. 2012a; Hii et al. 2012b; Halide & Ridd 2008; Xu et al. 2014).

Epidemiological Variables

Alarm Indicators: Probable and Hospitalised Cases

Epidemiological variables have already been used to predict outbreaks retrospectively, with some success (Halide & Ridd 2008). Of the epidemiological alarm indicators studied here, hospitalised cases and probable cases demonstrated the greatest potential for predictive capacity. Performance metrics were particularly good for the alarm indicator hospitalised cases in both Mexico and Dominican Republic, where sensitivities and PPVs were 92% and 45%, and 95% and 48% respectively (Figure 5.10). In those same countries, performance metrics were similarly high (91% and 40%, 92% and 45% respectively) where the alarm indicator was probable cases (Figure 5.9). Sensitivities were high in other countries, but PPV values were less than 26% in all cases (Figures 5.10 and 5.11).

By introducing a lag period it is possible to generate an outbreak probability for hospitalised cases as an alarm indicator during the historic period. This outbreak probability was used during the period of evaluation to predict outbreaks comprised of hospitalised incident cases. The high sensitivity of hospitalised cases as an alarm indicator suggests that incidence had been frequently above the alarm threshold during the historic period, resulting in many alarm periods that captured most outbreak periods during the period of evaluation. This is consistent with the global epidemiological picture of a recent rising trend in dengue incidence (World Health Organisation 2012a) and the epidemiology in these particular countries and regions at large (Mia et al. 2013; Banu et al. 2014; Dantés et al. 2014; Paixão et al. 2015; Anders et al. 2011; San Martin et al. 2010). That there were also roughly 1 in 2 false alarms for each outbreak period suggests that the alarm threshold might be too low in these countries, that outbreaks have occurred outside the normal seasons or that the alarm definition is not robust enough. Indeed, it would be possible to alter the alarm definition to n = 2 consecutive alarm signals, which could increase the PPV by decreasing the total number of alarm periods created. However, this could negatively impact sensitivity simultaneously. Notably, the PPV calculation did not include subsequent successful alarms that were recorded after the outbreak was already detected *i.e.* after the first successful alarm. Accordingly, this biased the PPV towards the null. Otherwise, the differences observed between countries in the PPV of the alarm indicators most likely reflect the context-dependent nature of dengue transmission, which has long been argued as a feature of dengue transmission (Brady et al. 2015; Scott & Morrison 2010; Karl et al. 2014). In both Dominican Republic and Mexico, it could be argued that the modest success achieved was because the period of evaluation reflected the patterns observed during the historic period, *i.e.* that dengue trends remained similar during both historic and evaluation periods, thereby resulting in fewer false alarm periods. However, these country differences could also be attributed to noisier country-specific datasets, given that the length of historic periods were relatively low when compared to similar forecasting models (Barbazan et al. 2002; Hii et al. 2012b; Lowe et al. 2014).

Detection of an increase in the number of hospitalised cases has little value as a prospective early warning alarm indicator. This is simply due to the reporting delay associated with such case definitions, coupled with hospitalisation occurring in a small proportion of cases, after a mean incubation period of anywhere between 3-10 days (Chan & Johansson 2012). On the other hand, where mandatory probable dengue case reporting is part of an active surveillance system, this alarm indicator could provide timely warnings of value to epidemiologists. Our analyses demonstrate some potential for this alarm indicator, though it appears to be context dependent. The evidence suggests that in certain countries, pre-outbreak surges in probable cases are quite common. Yet, there is a stark difference between the PPV values of two out of four countries (this variable could not be tested in Malaysia): in Brazil and Viet Nam, PPV values were half those recorded in Mexico and Dominican

Republic. This could be due to the presence of co-circulating infections with similar clinical presentations, such as Chikungunya or Zika viruses (Musso et al. 2015; Cardona-Ospina et al. 2015), which may be confounding probable dengue case diagnoses, or indeed because case definitions are less specific (or likely a combination of both) (Guzman et al. 2010; World Health Organisation 2012a).

In summary, while suspected or probable cases are notifiable within many existing disease surveillance systems (Runge-Ranzinger et al. 2014; Runge-Ranzinger et al. 2008), these data suggest that probable case data alone should not be used to predict outbreaks, although they could be used as an appropriate addition to other earlier alarm indicators, to confirm the increasing/ growing risk of dengue outbreaks for/during the following 1 – 12 weeks.

Alarm Indicator: Mean Age

Sensitivities using this alarm indicator were, on balance, moderately high (>65% in Mexico, Malaysia and Brazil), while PPV values ranged between 15-40%. In the context of this specific model, these results indicated that the predictive power of this alarm indicator is limited.

Theoretically, since population-level serotype shifts are known to fluctuate over periods of years (Reiner et al. 2014; Morrison et al. 2010; Rodrigues et al. 2011; Nisalak et al. 2003), thereby influencing the herd immunity of the age distribution of a population (Rodrigues et al. 2011), it should be possible to detect such changes through a proxy increase or decrease in the mean age of infection (Stoddard et al. 2014). Yet the limited success of this model in detecting such changes suggests that these population transmission dynamics are not very apparent. It may be possible to explain this by highlighting the limitations associated with mean age in the context of this particular study. Firstly, it was not possible to correlate the incident age distribution of dengue with serotype shifts to stratify the risk of infection among age groups, due to inconsistent data entry. Secondly, mean age was inconsistently calculated as either a function of probable or hospitalised cases, perhaps masking true associations that may have been more pronounced if the calculation had been standardised across countries. Finally, where the calculation of mean age was based on probable cases, the effect of poorer specificity within this case definition likely diluted any associations with the outbreak indicator ‘probable cases’, which may explain why all countries, excepting Viet Nam, generated lower PPV values when compared with the outbreak indicator hospitalised cases.

Despite these constraints, the potential for incident mean age as an alarm indicator remains. However, in order to achieve reliable results, it should be noted that probable case definitions are less accurate and thus co-incident data of related infections should be collected to control for any confounding effect. Thereafter, the true impact of predominant serotypes on shifting mean age can be properly explored, in conjunction with accurate reporting of serotype data.

Outbreak Indicators: Probable and hospitalised cases

Defining outbreaks using hospitalised cases, a common practice today (Badurdeen et al. 2013), broadly demonstrated significantly better predictions than using probable cases. It is reasonable to presume that the lower performance metrics associated with probable case definitions was likely the result of less specific dengue case definitions. Consequently, outbreak probabilities calculated during the historic period were less strongly associated with alarm indicators, thereby consistently reducing the performance metrics for each dataset accordingly. And yet, the utility of probable case definitions as outbreak indicators should not be ruled out, as similar trends seen between alarm indicators and hospitalised cases, were indeed also observed between alarm indicators and probable cases. Thus, when developing endemic channels and epidemic curves, this indicator could be used as a pragmatic solution to the reporting delay often associated with more specific outbreak indicators (World Health Organisation 2012a) (albeit increasing systematic error).

Meteorological Variables

Given their direct influence on vector population dynamics and on the extrinsic incubation period of the dengue virus, it is perhaps not surprising that meteorological variables have demonstrated potential as early warning alarm indicators, both in this study and various mathematical models also using field data (Johansson et al. 2009; Phung et al. 2015; Hii et al. 2012a). Here, we have demonstrated that certain meteorological variables, such as temperature, rainfall, and to a lesser extent, humidity, also hold potential as predictors of outbreak periods.

Comprehensive Datasets

Countries that had access to better meteorological datasets produced higher performance metrics (Figures 5.9 and 5.10). Conversely, both Malaysia and Dominican Republic datasets suffered from poor sensitivities, likely as a result of few data points spread disparately over wide geographic areas. This is important for two reasons: 1) fewer, widely interspersed data points failed to capture localised meteorological variation; and 2) intra-district spatial inconsistencies between meteorological metrics and outbreak indicators were also likely to have affected outbreak probabilities *i.e.* incident cases may have been registered in locations of high altitude or on the coast yet within the same district. As a consequence, weaker regressions during the historic period produced noisy associations that obscured any true trends.

Mean Temperature, Rainfall and Mean Humidity

Mean temperature generally outperformed both rainfall and humidity, both in terms of sensitivities and PPVs, with both Brazil and Mexico datasets recording the highest sensitivities (Figures 5.9 and 5.10). Such associations with temperature have been observed before in dengue, as well as in other vector borne diseases, often as a consequence of the effect on the development rate of the vector and the extrinsic incubation period of the pathogen (Christiansen-Jucht et al. 2014; Yi et al. 2003; Zhang et al. 2008; Pham et al. 2011). In particular, temperature variations are known to influence DENV replication, vector survival and larval development (Rabaa et al. 2013; Tun-Lin et al. 2000; Hugo et al. 2014; Racloz et al. 2012), while rainfall, or lack thereof, exhibits variable effects on the availability and suitability of breeding sites (Hii et al. 2012a; Ninphanomchai et al. 2014; Meza-Ballesta & Gónima 2014; Tran et al. 2010; Campbell et al. 2013). The variation observed between countries in this study, particularly with regard to rainfall and humidity, is similar to other research that has also reported context-dependent meteorological alarm indicators (Naish et al. 2014). For example, rainfall has also been described as associated with subsequent dengue outbreaks (Chen & Hsieh 2012; Johansson et al. 2009), and although it has not been strongly predictive in all locations, perhaps due to the greater influence of human behaviour in contributing to the urban vector ecosystem (Naish et al. 2014), it appears that here, too, such meteorological indicators vary in potential from one context to the next.

Certainly, some of the variation observed within this study might be attributable to land use, vegetation, altitude and indeed human behaviour (Betts et al. 2004; Morin et al. 2013) - data that were not readily available during the data capture process. At the same time, spatial smoothing effects might also be a contributory factor, as district sizes were not standardised between countries - working at coarser resolutions tends to obscure or weaken associations often present at finer spatial scales. Finally, one possible mechanism that may influence the degree to which meteorological variables affect dengue transmission, is the co-existence of inter-related meteorological variables present in a continuous, stable, optimal state.

While the differences in alarm indicator successes vary between country, this is not unexpected, due to the multiplicity of context-dependent transmission factors (Campbell et al. 2013) and the suggested effect of global warming on shifting spatial DENV transmission (Morin et al. 2013). Indeed, as climates and meteorological indicators start to change, historic trends may become less indicative of future observations (Morin et al. 2013), although, admittedly, a drastic short-term change in climate would be necessary to disrupt the trends used in this study. Nevertheless, short-term, minor, localised shifts, such as the El Nino Southern Oscillation (ENSO), may change weather enough to impact dengue transmission. Indeed, the results seen here may be a product of varied fluctuating meteorological conditions during a small window of time, which may not necessarily reflect truer, long-term trends. Certainly, from a prospective point of view and in consideration of future DENV trends, predicted global and local changes in the climate and weather, as caused by events such as ENSO, present a fundamental challenge to dengue forecasting and prediction (Morin et al. 2013).

Defining Outbreaks

While not without limitation, the results demonstrated that the Endemic Channel remains an operationally useful and simple aid, primarily because of its ability to clearly demarcate thresholds based on simple summary statistics. However, the major limitation, as demonstrated within this study, is the use of a generic Endemic Channel *i.e.* one that is used with a standardised 2*SD. Altering the z-value, hence defining outbreaks, can have dramatic implications on the number of outbreaks identified. Worldwide, a standard deviation of 2 is used to capture 95% of the variation in dengue incidence about the mean. This provides a threshold above which the number of cases is higher than historically normal incidence, and so remains a rational metric. However, it is important to identify that dengue incidence fluctuates on an inter-annual basis, and at any one time, is influenced potentially by numerous interacting variables that may not reflect historical patterns of incidence. Indeed, this also means that historic incidence may not accord with present or future transmission. This is further compounded in our model as associations are built on a relatively coarse countrywide scale, which obscures any regional or local spatial variation in transmission (Brady et al. 2015). In light of this, the use of 2SD becomes questionable.

How should dengue outbreaks be defined? In this study, we altered z-values to improve the success of detection, rather than consider the operational or financial implications of changing outbreak definitions, especially in a prospective capacity. This neglected the importance of accurate metrics in these contexts; low z-values resulted in outbreaks that were often infrequent, long and protracted in nature, requiring resource-intensive responses. As the z-value gradually increased, only the highest magnitude peaks were captured (Figure 5.2). In this scenario, standardised thresholds failed to distinguish between certain types of outbreak, e.g. propagated vs. point source, of which dengue is most certainly the former. Dengue transmission, characterised by various peaks in incidence, is a function of variable intrinsic and extrinsic incubation periods amongst populations with varied herd immunity. Therefore, based on this kind of transmission, as one increases the z-value, there will come a point at which a greater frequency of distinct outbreaks is recorded, resulting in shorter duration but greater frequency outbreak responses (Figure 5.2). Such pendulum swings in a predictive capacity between the presence and absence of outbreaks would likely cause a greater administrative burden on surveillance and health infrastructure, as well as increase confusion and mistrust amongst the local population due to perceived unreliable forecasting (Rosenbaum 2015). Of course, eventually, as the z-value continues to rise, the number of outbreaks falls away. But this conundrum is clearly important from a both modelling and operational perspective, and should be considered in any future costing/ prediction of dengue outbreaks. Finally, future outbreak definitions might concentrate on capturing specific time points of an outbreak *i.e.* using the incident mean difference between weeks to capture the earliest stages, as this is when interventions would be most effective, both clinically and financially.

Temporal Associations between Alarm and Outbreak Indicators

Mean Lag Time

Mean lag time fell consistently as the alarm threshold increased (Table 5.1). To explain this, first it is important to understand the relationship between the mean lag time and the alarm threshold. It is clear that lower alarm thresholds capture a greater number of alarm signals/ periods within the lag period. Notably, over time, any signals in addition to those required to form an alarm period that occur after the first observation of an alarm period, form alarm periods that are not considered in PPV calculations. Consequently, alarm periods that would have occurred at higher outbreak probabilities and were temporally closer to the outbreak, were not recorded, in favour of lower probability, more distant alarm signals/ periods. As the threshold increases, these greater probability alarm signals become the only signals that remain to be detected, thus alarm periods are formed later within the lag period (closer to the outbreak), thus reducing the mean lag time. All things being equal, this demonstrates that alarm signals closer to the outbreak are usually greater in magnitude where the trend is positive, indicating that changes above the historical norm occur more frequently as the outbreak is approached. However, as just described, it is important to note that these associations are at least somewhat influenced by the model parameterisation, rather than any necessarily true temporal associations between alarm and outbreak indicators *i.e.* increasing the z-value and changing the *n* required to form alarm signals alters the mean lag time, irrespective of any true temporal associations between alarm and outbreak indicators. Thus, new approaches are needed to generate more reliable metrics to truly identify and quantify these associations.

Timely Outbreak Detection

Using 2 or 3 alarm/ outbreak signals to define alarm/ outbreak periods produced the highest outcome metrics, while there was little difference between these two multipliers across all indicators (Figures 5.9-5.12). As demonstrated previously, altering this multiplier can increase or decrease the outbreak detection times, which is particularly important in a prospective capacity. With this in mind, it was important to keep *n* relatively low (Figure 5.5).

Working with the moving average delays the anticipated outbreak pattern by delaying the increase and postponing the decrease in incidence. This is a method that could be altered in future model iterations, and indeed if shortened from the current 6+1+6 to 3+1+3, would likely decrease the time necessary to form outbreak periods, which is particularly important in a prospective capacity. However one would need to ensure that outbreaks using this altered approach are not the result of increased noise among the data.

Alarm periods formed during an outbreak were discarded, and given that the shortest lag period used was 1-12 weeks, it is feasible that alarms towards the end of the current outbreak period were indeed correlated with later outbreaks. One possible method to overcome this would be to shorten the lag period considerably. However, this would have decreased the chance of detecting alarm signals, thereby reducing the sensitivity of the model.

Multivariate Analyses

In combining the strongest singly predictive alarm indicators, the model could not improve upon or indeed reproduce the performance metrics observed during univariate analyses. The results were favourable with the Mexico dataset but failed to outperform univariate analyses using either the Brazil or Dominican Republic datasets (Figure 5.14). That performance metrics did not improve is surprising, but this may be due to poorer outbreak probabilities, given that multivariate analyses combined alarm indicators to produce one outbreak probability, rather than using the datasets to produce two independent outbreak probabilities. Using the latter approach would have provided two opportunities to detect the same outbreak and is under consideration as an additional avenue of exploration in future iterations.

Candidate Alarm Indicators

In addition to the alarm indicators explored within this study, there is increasing evidence that novel indicators may prove valuable in forecasting dengue outbreaks. Internet-based trending metrics can warn of forthcoming outbreaks, with evidence suggesting that these data might be useful for predicting dramatic surges in dengue incidence (Gluskin et al. 2014), using both search query data (Chan et al. 2011; Althouse et al. 2011) and social media trends (Chunara et al. 2012), although the latter was not evaluated in relation to dengue. Other avenues of exploration could also include the use of alternate summary statistics for those alarm and outbreak indicators already explored within this study, such as the diurnal temperature range instead of the mean temperature, or cumulative mean instead of the moving mean (Liu-Helmersson et al. 2014; Brady et al. 2015). And as the use of GIS-based and remotely sensed data capture becomes increasingly prevalent, spatial analyses and prediction based on the clustering nature of dengue and geo-referencing of alarm indicators should enable scientists to better pinpoint potential high risk transmission areas at smaller spatial scales (Hernández-Ávila et al. 2013; Louis et al. 2014).

LIMITATIONS

Data Limitations

Inconsistent data collection and missing data almost certainly affected the quality of datasets, especially with regard to entomological indices. Entomological indices were generated on varied temporal and/ or spatial scales in different countries, resulting in a mismatch with the outbreak indicators. Accordingly, these alarm indicators could not be fairly evaluated.

Climate data were obtained either from local weather stations or from published websites, excepting Viet Nam, where access to data could not be arranged. Also, it was not possible to obtain district-specific climate data in all cases, which masked variations that may have increased the predictive capacity of the variable in question.

The following additional limitations in the routine surveillance data were observed:

- Temporal variation (monthly timescale observed for some entomological indicators)
- Spatial variation (data, especially meteorological, were sometimes only available at coarser resolutions)
- Paucity/ absence of data/ variables
- Varied data sources (independent online systems)
- Multiple non-verifiable data sources
- Random (inconsistent) sampling (particularly entomological indices)
- Annual data entered only on one date rather than each week of the year
- As indicated above, mean age calculations were inconsistent between countries

Model Considerations

The moving average and regression probabilities calculated during the historic period were reliant upon a relatively low number of years (<3) of historic data, in contrast to others forecasting models (Barbazan et al. 2002; Hii et al. 2012a; Lowe et al. 2014). Using a greater number of historic years would generate a more stable mean and outbreak probability.

Outbreak probabilities for alarm indicators were based on countrywide associations, which invariably changed any variation observed at the district level, potentially underestimating true probabilities. Also, logistic regression functions could not control for co-linearity between alarm variables (Racloz et al. 2012), and thus certain inter-related alarm indicators were not explored. To this extent, the model could be improved.

The study methods yielded z-values of 1.25 as most appropriate for the highest sensitivities when used systematically across large spatial units (country). In this case, lower z-values than 2.0 are explained by the inclusion of epidemic years during the historic period, which would give larger standard deviations compared to most other studies that excluded epidemic years (Badurdeen et al. 2013). Combining this z-value with an outbreak probability of 0.12 yielded practical sensitivities/ PPVs, although the authors note that these values would likely benefit from minor alterations to suit individual spatial units in any future prospective investigations.

Some variables, in particular temperature, have been known to show non-monotonic relations concerning mosquito and viral replication (Morin et al. 2013; Tun-Lin et al. 2000; Rueda et al. 1990), however these effects were not adequately captured in the current model. Where possible, such relations will be considered in future model iterations.

CONCLUSIONS AND RECOMMENDATIONS

The findings reported here suggest that the Shewhart Method – a relatively simple approach - is a viable technique that can be used retrospectively, and potentially prospectively, to detect dengue outbreaks using alarm indicators with an attributed lag time. This approach builds on earlier observations that utilised multiple alarm indicators on similar spatial/ temporal scales (Racloz et al. 2012), and combined prior theoretical observations into a practical model (Johansson et al. 2009). However, there is emerging evidence of alternative models that may be used for time series datasets, in particular, the LASSO (least absolute shrinkage and selection) method. Evidence has suggested that forecasts using this approach may be more accurate than the approaches used in this study (Shi et al. 2015), although the LASSO approach requires particularly detailed time series data. In this respect, due to the observed paucity in district datasets throughout this study, the LASSO method would not have been an appropriate evaluative approach.

Of the epidemiological alarm indicators studied, the number of probable cases showed greatest predictive potential and should be routinely captured during active surveillance systems for use in predictive models. Increases in this metric may provide advance warning of increasing dengue outbreak risks in subsequent time periods (in this study, 1 – 12 weeks). In contrast, the mean age of dengue cases requires further validation as a potential indicator.

The use of hospitalised cases as the outbreak indicator produced higher model performance metrics with alarm indicators vs. probable cases and alarm indicators. This is likely the result of the more specific case definition of hospitalised cases. That said, although associations between alarm indicators and probable cases were weaker, the same trends were observed as those between alarm indicators and hospitalised cases. This justifies the use of probable cases as an outbreak indicator in a prospective context where there is significant delay associated with hospitalised cases.

Meteorological alarm indicators were more powerful predictors of outbreak periods in both Mexico and Brazil than other countries, likely due to more frequent spatial data points and accurate spatial correlations with outbreak indicators. Therefore, where spatial meteorological data are discordant with the spatial area of analysis, interpolation techniques should be used to mitigate the problems experienced in this study. Indeed, given the widespread availability of temperature and humidity data, dengue surveillance programmes should routinely record these metrics in order to detect any sustained abnormal changes. While rainfall data are more difficult to capture, these data are still a useful metric if they are spatially correlated with incidence.

Exploratory analyses of the value of entomological indices as predictors of epidemic dengue transmission are still required.

In spite of the limitations it is clear that, in the absence of process-based models, predictive dengue modelling must be based on available retrospective datasets, validated across multiple contexts. Equally, datasets compiled from mandatory electronic reporting and standardised surveillance systems will greatly improve the quality of datasets by limiting misreporting and bias. Though modifications to improve the PPV and other aspects of the model are needed, the method used in this study has been shown as suitable to identify potential alarm indicators that sensitively predict forthcoming outbreaks. The model could also be simply transformed into a real-time, user-friendly operational tool to identify at-risk areas in order to allocate resources more efficiently (Racloz et al. 2012). At the time of writing, the model is deployed in a predictive capacity across 3 dengue-endemic countries, with initial results expected in the latter part of 2016.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The data, results and conclusions of the chapters presented in this thesis contribute to a greater understanding of dengue epidemiology, in particular vector surveillance, control and early warning systems. Together, they indicate a roadmap for future research avenues and highlighted some of the current limitations within the field of dengue. It is hoped that these chapters will drive the research agenda and future policy for the control and response to dengue endemicity and epidemics worldwide.

Vector Surveillance and Early Warning Systems

Of the variables that are currently quantifiable, entomological metrics have neither been proven nor disproven as valuable surveillance metrics. Given that this practice is either passively or actively performed in many countries worldwide (Harrington et al. 2013; Horstick et al. 2010; Runge-Ranzinger et al. 2014), it should not be discontinued until further research emerges. Currently, there are many disconnects in our knowledge of entomological transmission dynamics, many of which exist between the various metrics produced for vector abundance: immature indices do not correlate with adult indices; cryptic sites cannot be quantified; adult mosquito abundance metrics do not correlate with the proportion of those infected; the age of circulating mosquito populations may affect resulting transmission dynamics; infectivity rates between mosquito and human are not established (Focks 2004; Scott & Morrison 2010). And yet clearly the absence of mosquitoes prevents mosquito-borne transmission, but to obtain true estimates of the abundance necessary to maintain both endemic and epidemic dengue transmission, localised estimates must account for spatiotemporal variation in abundance and should be used to generate baseline data upon which alarm signals can be built (Chang et al. 2015). Indeed, localised metrics that focus on the adult mosquito, and perhaps mosquito infectivity rates, may circumvent many of the problems known with immature stage metrics and the presence of cryptic breeding sites. Although uncommon, diagnostic tools that quantify mosquito infectivity rates are increasingly available in the field (Pal et al. 2015; Voge et al. 2013), and it is likely that with updated capture methods (Vazquez-Prokopec et al. 2009), these infectivity surveys will become standardised tools to quantify prevailing dengue risk. In Chapters 2 and 5, identifying predictive entomological indicators among datasets was not possible due to incomplete data, but only with continued data capture can these datasets improve and thereby eliminate the knowledge gap between these indices and dengue risk (Focks 2004).

Fortunately, modelling of climatic and epidemiological variables was more successful, as fluctuations in mean temperature and probable dengue cases proved highly sensitive to forthcoming outbreaks. And yet, a high combination of sensitivity and positive predictive value remained elusive, even if changes to the formulae for these calculations will likely improve these outputs. These results are indicative of the mixed contemporary picture for dengue EWS: recent efforts show that meteorological and epidemiological variables ably predict outbreaks (Ninphanomchai et al. 2014; Hii et al. 2012a), yet there is also contrasting data from non-endemic settings (Chang et al. 2015), where few associations between alarm variables and outbreaks were found. Still, elsewhere, EWS have been evaluated in real-time for West Nile (Manore et al. 2014) and influenza (Waziri et al. 2014), although sensitivity and positive predictive values were relatively low, which would lead to many false alarms. However, this has not deterred the European Centre for Disease Control from establishing a surveillance unit with an active programme in developing early warning systems (Semenza 2015). What emerges from this picture is that still there remain many interactions that influence dengue transmission not adequately captured by this model and others elsewhere, and may explain why recent research suggests that outbreaks are implicitly variable (Brady et al. 2015).

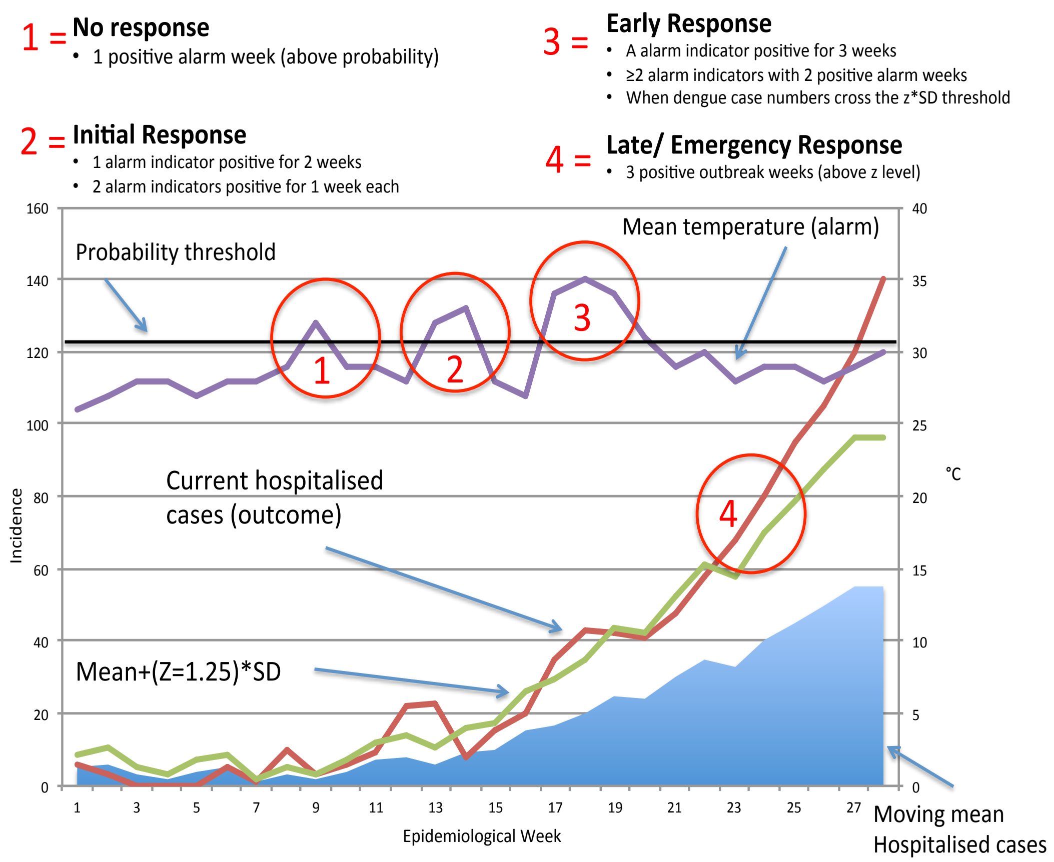
Increasingly, early warning systems are used for the prediction of infectious disease outbreaks, ranging from mumps and influenza to vector borne diseases such as malaria and West Nile (Semenza 2015; Sudre et al. 2013; Chaintoutis et al. 2014; Manore et al. 2014; Hughes et al. 2015; Fan et al. 2014). In these cases, other alarm signals were used, such as the seroconversion rate in backyard chickens prior to

West Nile virus outbreaks (Chaintoutis et al. 2014). The obvious parallel with dengue is the mosquito infection rate, which might provide advanced warning of subsequent elevated transmission amongst the human population, but for reasons previously discussed, limitations still apply to this technique.

Nonetheless, clearly, the ability to predict such hazardous events has distinct advantages for human populations, and yet these predictions are often clouded by uncertainty. A large part of the uncertainty for disease surveillance is the presence of existing covariates that influence risk or transmission dynamics, and yet are either unknown or cannot be measured. Indeed, complex interactions between these covariates somewhat confound prediction (Louis et al. 2014), in part due to algorithms failing to capture non-monotonic relations between variables (May & Bigelow 2005). Strong examples of the paucity in knowledge can be found in both transmission dynamics and the social cost of dengue. While burden estimates include the number of asymptomatic patients (Bhatt et al. 2013), precisely how they contribute to outbreak transmission still remains unknown (Chastel 2012). Fortunately it is now possible to identify these individuals in the field (Yeo et al. 2015) and adequately quantify them, which should lead to increased knowledge of their role in dengue transmission. In addition, the social cost of dengue-related workplace/ school absenteeism remains a mystery. Those at higher risk of dengue are often from poorer backgrounds (World Health Organisation 2009) while children in particular are at higher risk of severe dengue (Guzman et al. 2002). It follows then that infected children may fall behind on schoolwork, while employees might need to take unpaid leave. In both examples, downstream effects may occur that continue the cycle of poverty in a negative feedback loop. While these aspects remain difficult to quantify, the existence of absenteeism has been documented in a number of dengue endemic communities (Halasa et al. 2012; Lawpoolsri et al. 2014) and has been identified as coincident with dengue incident data (Lawpoolsri et al. 2014), and while EWS models based on this metric have been forthcoming, the reality is that lead time may be too short to provide meaningful alerts (Fan et al. 2014).

Possible routes forward for EWS will incorporate the myriad of known qualitative and quantitative covariates central to dengue transmission in a complete ESW framework (Louis et al. 2014). Importantly, a sole reliance on quantitative aspects would be foolhardy, given that short term changes among the population (that may be difficult to immediately quantify), such as short-term population movement and a reduction in herd immunity (via an influx of susceptible individuals), also increase outbreak risk (Lowe et al. 2014). Staged response mechanisms based on the severity of disease is one way forward. Various responses could be indicated by moving from one alert level to the next, driven by the presence of risk indicators/ modifiers that would, in turn, initiate a greater response. Indeed, a variant of this approach is currently employed in four countries, where risk indicators or alarm signals are used to quantify risk based on regression probabilities (Figure 6.1).

Figure 6.1. Staged Response System. Increasingly resource intensive interventions are implemented as the number of alarm signals increases.



Incorporating each of the abovementioned considerations in an algorithm alongside demographic and geographic risk factors will allow transmission estimates, such as the reproductive number (R_1), to better reflect dengue transmission (Louis et al. 2014). Ultimately, it is clear that increasing granularity is necessary to account for local spatiotemporal transmission dynamics that may vary from place to place (Van Panhuis et al. 2015). Equally, as the true cost of dengue, both economic and social becomes available at finer spatial scales, forecasting modellers can be better evaluated. And finally, improved transmission estimates based on more accurate dengue transmission dynamics and adequate weighting to reflect the importance of individual quantitative and qualitative risk indicators at a localised level (Chang et al. 2015) will likely result in an improvement to surveillance and early warning systems for dengue.

Vaccines and Vector Control: A Crowded Picture?

Fortunately, as demonstrated in Chapter 3, some evidence for effectiveness of vector control is emerging, especially for community-based campaigns. Interventions at this level are effective against the proliferation of dengue vectors, while recent evidence has emerged that such approaches can also impact dengue incidence (Andersson et al. 2015). But more evidence is needed. Given the upward trend of dengue incidence today and the widespread use of poorly evaluated vector control tools, there is a strong argument for an expert committee or panel to produce guidelines for undertaking vector control trials. Indeed, progress has begun with the availability of trial guidelines and identification of outstanding knowledge gaps already produced (Achee et al. 2015; Wilson et al. 2015). With this knowledge it should be possible to coordinate vector control trials to produce a comprehensive series of publications that underpin the use of vector control internationally. Indeed, the same approach has been taken when evaluating the DENV vaccine produced by Sanofi Pasteur (Da Costa et al. 2014), and if both interventions are perceived as complementary in the future, surely evidence of effectiveness should exist for both. But there is a danger that existing vector control tools will be displaced in favour of recently evaluated novel mosquito manipulation techniques, without fair trial. A unified evaluative approach for the most promising existing and novel approaches is especially pressing as the associated impact of dengue outbreaks continues to rise steeply in affected communities (Hotez et al. 2014; L'Azou et al. 2014). In light of this, the dengue community cannot afford to underuse any available strategy. Indeed it would be a failure by all stakeholders if these steps were not prioritised to distinguish between the truly effective and ineffective vector control tools.

Using vaccines as a form of outbreak control is historically successful where ring vaccination is feasible and appropriate (Xu et al. 2014), however vaccine introduction for dengue in emergency settings is unrealistic for the current Sanofi Pasteur vaccine candidate, as 2 boosters after the initial inoculation are required for complete protection (Capeding et al. 2014). As vaccine development progresses, researchers are currently weighing up the role that vector control might still play. Indeed, some in the field believe that even when an effective vaccine is proven, integrated programmes that include both vaccine and vector control will be paramount (Achee et al. 2015). Barriers to adoption, delivery, coverage and cost, community accessibility, deployment, cold-chain logistics and booster doses will all need consideration when deploying any efficacious vaccine (Douglas et al. 2013; Lam et al. 2011). Furthermore, localised urban geography, safety and stability among such populations will also play a key role, as well as economic evaluations that will ensure that governments utilise the most cost-effective interventions (Lam et al. 2011). The prospect of synergistic, effective dengue control should encourage the international community to prioritise combined vector control and vaccine deployment models to ensure best practice when the situation arises.

Insecticides for Dengue Vectors

Integrated vector control methods used both in trials and operationally is a natural progression for vector borne diseases (Golding et al. 2015). In this regard, insecticides that broadly target all diseases are advantageous over vector-specific tools. However, haematophagous insects are becoming increasingly resistant to all classes of insecticides, in particular pyrethroids and organophosphates (Ranson et al. 2010; Rodriguez et al. 2007). In light of this, novel tools, such as RIDL techniques (Harris et al. 2012) and *Wolbachia* (Lambrechts et al. 2015), are being developed that move away from insecticide-based control. Equally, community-based campaigns that are primarily mechanical in nature are gaining traction as effective dengue control tools (Andersson et al. 2015). Such advances are important for ecosystems, as well as long-term human health. A reliance on single, class-specific pesticides rather than biological, mechanical or genetic methods will only continue to produce resistance mechanisms in the target vectors. Certainly, it is possible for insecticide resistance to be properly managed, with guidelines present in the literature (Ranson et al. 2010; World Health Organisation 2012a) but given the extent to which insecticides are currently arbitrarily used in all forms of vector control and agriculture, the status quo is perhaps unlikely to change unless governmental pressure is forthcoming, arising from strong research supporting a change in insecticide resistance management. Until combination insecticides are developed that move away from a reliance on single-class insecticides, it might be prudent to move towards non-insecticidal evidence-based control measures, such as combination interventions involving community-based clean-up campaigns, house screening and a general improvement in house structure and reliable water supplies, which may be directly or indirectly effective against dengue vectors.

Knowledge Gaps, Monitoring and Evaluation

Dengue vector control trials are important for the evaluation of control tools. As observed in Chapters 2 and 3, outcomes should include vector metrics and dengue incidence. This allows correlations between a drop in vector abundance and dengue incidence to be quantified. In addition, a move towards routine electronic data capture for all metrics will greatly improve the quality of reporting and any subsequent calculations for transmission dynamics and forecasting, as noted in Chapters 2, 3 and 5.

Since dengue vector mosquito species are diurnally active, virus transmission is not limited to the home *e.g.* exposure is apparent in many social environments, including schools, workplaces, markets and places of worship (Reiner et al. 2014); consequently, cluster designs are routinely employed to discourage any mosquito interaction between control and intervention groups (Kroeger et al. 2006; Lenhart et al. 2013). However, such designs cannot control for the movement of people. To overcome this, tracking human movement has been the subject of recent research (Stoddard et al. 2009; Stoddard et al. 2013), and although expensive and laborious, these data contribute to estimates of the force of infection, the rate at which susceptible humans become infected (Reiner et al. 2014). Clearly, it is not possible to estimate this metric without prior knowledge of the index case and the use of contact tracing, given the varied exposures and seropositivity status of individuals among the community. These investigations are particularly useful as they also overcome case underreporting, a major problem in endemic countries such as Sri Lanka, where ratios of 100:1 among infants (infected infants to notified infant cases) and 30:1 among children were detected (Tam et al. 2013). Such investigations also capture the presence of asymptomatic dengue cases, which when compared with symptomatic patients can be reportedly up to ratios of 13:1 (but as low as 0.9:1) (Chastel 2012; Balmaseda et al. 2006; Endy 2002) and who are believed to be a significant factor in dengue transmission (Leite et al. 2014). Where the ratio of asymptomatic to symptomatic cases is high, the epidemiological role of such 'silent spreaders' is likely to be dramatic, and cause both greater model uncertainty (in the case of Chapter 5, weaken or delay associations with predictor variables) and likely result in limited operational interventions, as the true magnitude of an outbreak would remain underreported. To account for these dynamics, perhaps a move towards observational studies, such as case-control designs, to evaluate the role of human movement, asymptomatic cases and underreporting is warranted, which would provide greater understanding of the complex interplay of transmission dynamics and how interventions in the form of vector control and vaccines may impact endemic and epidemic dengue transmission (Reiner et al. 2014).

Epidemiological trials are essential for population-based health metrics and mass interventions. While trials are widespread, in the past these trials were not widely known and accessible. This created three problems: 1) low accountability towards trial completion 2) possible cross-funding for similar interventional research that was already underway elsewhere 3) limited input from persons external to the trial who may have valuable input to improve trial design. Fortunately, trials can be routinely registered via the EU (Chang et al. 2015; European Medicines Agency 2015), local government (National Institutes of Health 2015) and/ or independent online portals (ISRCTN Registry 2015) and protocols are published in associated peer reviewed journals (Trial Journal 2015), which provide greater accountability, transparency and the possibility to refine trial designs before mistakes are made in the field. This approach is becoming mainstream, but there still is no external pressure on failed trials to publish what material they have. The systemic problem of selective publishing in the pharmaceutical industry has led to a growing movement within large corporates to publish all trial results (GlaxoSmithKline 2015). Perhaps a similar obligation toward the publication of failed trial results would further strengthen accountability and transparency, and also ensure that the field can learn from others' mistakes.

CONCLUSIONS

Dengue control can only progress with significant research into the fundamental understanding of the complex transmission dynamics that underpin endemic and epidemic dengue transmission. Surveillance methodologies used to capture these covariate data require standardisation, in particular, entomological metrics for surveillance, while further funding is essential if vector control tools are to be adequately evaluated in the field. Indeed, each year, hundreds of vector control campaigns are conducted by programme managers worldwide, yet very few are adequately evaluated. Each of these is a missed opportunity. Fortunately, guidelines for the proficient monitoring and evaluation of these tools are emerging (Wilson et al. 2015; Achee et al. 2015), which should ensure that evidence of effectiveness of vector control is forthcoming. This evidence will feed into cost-effectiveness calculations and provide a foundation for the necessary coexistence of vaccine and vector control delivery. Equally, these data can also be used to inform the next iteration of early warning systems, which crucially rely on accurate and scientifically observed interactions at the biological and population level. Finally, the combination of effective surveillance, early warning and vector control need to be adequately assessed in rigorous epidemiological trials. In line with increasing transparency in the pharmaceutical sector, failed trials should also be collated in a repository to further strengthen accountability, transparency and positive feedback mechanisms. Together, these approaches will ensure that dengue and indeed other neglected tropical diseases can move towards effective surveillance, management and control, with a view to elimination and eradication.

REFERENCES

Abreu F.V.S. et al., 2015. Influence of breeding site availability on the oviposition behaviour of Aedes aegypti. *Memorio Instituto Oswaldo Cruz*, 110(5), pp.669-676.

Achee, N. et al., 2012. Identifying the effective concentration for spatial repellency of the dengue vector Aedes aegypti. *Parasites & Vectors*, 5, pp.300-300.

Achee, N. et al., 2015. A critical assessment of vector control for dengue prevention. *PLoS Neglected Tropical Diseases*, 9(5), pp.e0003655.

Achee, N.L. et al., 2012. Spatial repellents: from discovery and development to evidence-based validation. *Malaria Journal*, 11(1), pp.1-1.

Achee, N.L. et al., 2015. A critical Assessment of vector control for dengue prevention. *PLoS Neglected Tropical Diseases*, 9(5), pp.e0003655.

Ahmed, N.H. & Broor, S., 2014. Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase. *Journal of Vector Borne Diseases*, 51(3), pp.194-199.

Alirol, E. et al., 2011. Urbanisation and infectious diseases in a globalised world. The *Lancet Infectious Diseases*, 11(2), pp.131-141.

Alphey, L. & Alphey, N., 2014. Five things to know about genetically modified (GM) insects for vector control L. *PLoS Pathogens*, 10(3), pp.e1003909.

Alphey, L. & Beech, C., 2012. Appropriate regulation of GM insects. *PLoS Neglected Tropical Diseases*, 6(1), pp.e1496.

Althouse, B.M., Ng, Y.Y. & Cummings, D.A.T., 2011. Prediction of dengue incidence using search query surveillance. *PLoS Neglected Tropical Diseases*, 5(8), pp.e1258.

Alvarez, L.C. et al., 2014. Susceptibility status of Aedes aegypti (L.) (Diptera: Culicidae) to temephos in Venezuela. *Pest Management Science*, 70(8), pp.1262-1266.

Anders KL & Hay SI (2012) Lessons from malaria control to help meet the rising challenge of dengue. *Lancet Infectious Diseases*, 12(12) pp.977-984. doi: 10.1016/S1473-3099(12)70246-3.

Anders, K.L. et al., 2011. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. *American Journal Of Tropical Medicine And Hygiene*, 84(1), pp.127-134.

Andersson, N. et al., 2015. Evidence based community mobilization for dengue prevention in Nicaragua and Mexico (Camino Verde, the Green Way): cluster randomized controlled trial. *British Medical Journal* (Clinical research ed), 351, pp.3267.

Andrade C.F. & Branco Júnior A.C, 1990. Methods for field detection of resistance to temephos in simuliids. Larval esterase level and topical application of the insecticide to adults. *Memorio Instituto Oswaldo Cruz*, 85, pp.291-297.

Andraud, M. et al., 2012. Dynamic epidemiological models for dengue transmission: A systematic review of structural approaches. *PloS One*, 7(11), pp.e49085.

Añez, G. & Rios, M., 2013. Dengue in the United States of America: A worsening scenario? *BioMed Research International*, 2013(3), pp.1-13.

National Institutes of Health, 2015. Clinical Trials. [online] Available at: https://clinicaltrials.gov [Accessed October 23, 2015].

European Medicines Agency, 2015. EU Clinical Trials Register. [online] Available at: https://www.clinicaltrialsregister.eu [Accessed October 23, 2015].

GlaxoSmithKline, 2015. GSK Clinical Study Register. [online] Available at: http://www.gsk-clinicalstudyregister.com [Accessed October 23, 2015].

ISRCTN Registry, 2015. [online] Available at: http://www.isrctn.com [Accessed October 23, 2015].

Stata Corp, 2015. STATA/ SE 13.1 for Mac. Texas: Stata Corp.

Trials Journal, 2015. [online] Available at: http://www.trialsjournal.com [Accessed October 23, 2015].

TuTiempo, 2015 Tutiempo Network, S.L. [online] Available at: http://en.tutiempo.net [Accessed November 1, 2014].

Wunderground, 2015. The Weather Channel, LLC. [online] Available at: http://www.wunderground.com [Accessed November 1, 2014].

Antman, E.M. et al., 1992. A comparison of results of meta-analyses of randomized control trials and recommendations of clinical experts. Treatments for myocardial infarction. *Journal of the American Medical Association*, 268(2), pp.240-248.

Armstrong, R., et al., 2007. Guidelines for Systematic Reviews of Health Promotion and Public Health Interventions. Version 2. Australia: Melbourne University.

Arunachalam, N. et al., 2012. Community-based control of Aedes aegypti by adoption of eco-health methods in Chennai City, India. *Pathogens and Global Health*, 106(8), pp.488-496.

Ávila Montes, G.A. et al., 2004. Evaluation of an educational module on dengue and Aedes aegypti for schoolchildren in Honduras. *Revista Panamericana de Salud Pública*, 16(2), pp.84-94.

Ayorinde, A. et al., 2015. The insecticide susceptibility status of Aedes aegypti (Diptera: Culicidae) in farm and nonfarm Sites of Lagos state, Nigeria. *Journal of Insect Science*, 15, pp.75.

Azmawati, M.N., Aniza, I. & Ali, M., 2013. Evaluation of communication for behavioral impact (COMBI) program in dengue prevention: A qualitative and quantitative study in Selangor, Malaysia. *Iranian Journal of Public Health*, 42(5), pp.538-539.

Badurdeen, S. et al., 2013. Sharing experiences: towards an evidence based model of dengue surveillance and outbreak response in Latin America and Asia. *BMC Public Health*, 13(1), pp.1-1.

Balmaseda, A. et al., 2006. High seroprevalence of antibodies against dengue virus in a prospective study of schoolchildren in Managua, Nicaragua. *Tropical Medicine & International Health*, 11(6), pp.935-942.

Baly, A. et al., 2009. Cost-effectiveness of a community-based approach intertwined with a vertical Aedes control program. *American Journal Of Tropical Medicine & Hygiene*, 81(1), pp.88-93.

Banu, S. et al., 2014. Dynamic spatiotemporal trends of dengue transmission in the Asia-Pacific region, 1955–2004. *PloS One*, 9(2), pp.e89440.

Barbazan, P., Yoksan, S. & Gonzalez, J.-P., 2002. Dengue hemorrhagic fever epidemiology in Thailand: description and forecasting of epidemics. *Microbes & Infection*, 4(7), pp.699-705.

Barrera, R., 2011. Spatial stability of adult Aedes aegypti populations. American *Journal of Tropical Medicine & Hygiene*, 85, pp.1087-1092 doi:10.4269/ajtmh.2011.11-0381.

Barrera, R. Amador, M. & MacKay, A.J., 2011. Population dynamics of Aedes aegypti and dengue as influenced by weather and human behavior in San Juan, Puerto Rico. *PLoS Neglected Tropical Diseases*, 5(12), pp.e1378. doi:10.1371/journal.pntd.0001378.

Barrera, R. et al., 2008. Unusual productivity of Aedes aegypti in septic tanks and its implications for dengue control. *Medical Veterinary Entomology*, 22, pp.62-69. doi: 10.1111/j.1365-2915.2008.00720.x.

Barrera, R., Mackay, A.J. & Amador, M., 2013. An improved autocidal gravid ovitrap for the control and surveillance of Aedes aegypti. *Parasites & Vectors*, 6, pp.225. doi: 10.1186/1756-3305-6-225.

Beatty, M.E. et al., 2010. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas dengue prevention boards. *PLoS Neglected Tropical Diseases*, 4(11), pp.e890-e890.

Beltrame, A. et al., 2007. Imported chikungunya infection, Italy. *Emerging Infectious Diseases*, 13(8), pp.1264-1266.

Benedict, M.Q. et al., 2007. Spread of the tiger: Global risk of invasion by the mosquito Aedes albopictus. *Vector-Borne & Zoonotic Diseases,* 7(1), pp.76-85.

Bernier, U.R. et al., 2015. Laboratory studies of Aedes aegypti attraction to ketones, sulfides, and primary chloroalkanes tested alone and in combination with L-lactic acid. *Journal of the American Mosquito Control Association*, 31(1), pp.63-70.

Betts, R.A. et al., 2004. The role of ecosystem-atmosphere interactions in simulated Amazonian precipitation decrease and forest dieback under global climate warming. *Theoretical and Applied Climatology*, 78, pp.1-3.

Bhatt, S., et al., 2013. The global distribution and burden of dengue. *Nature*, 496(7446), pp.504-7.

Borsboom, G. et al., 2003. Impact of ivermectin on onchocerciasis transmission: assessing the empirical evidence that repeated ivermectin mass treatments may lead to elimination/ eradication in West Africa. *Filaria Journal*, 2(1), pp.8.

Brady, O.J. et al., 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Neglected Tropical Diseases*, 6(8), pp.e1760.

Brady, O.J. et al., 2014. Global temperature constraints on Aedes aegypti and Ae. albopictus persistence and competence for dengue virus transmission. *Parasites & Vectors*, 7(1), pp.338.

Brady, O.J. et al., 2015. Dengue disease outbreak definitions are implicitly variable. *Epidemics*, 11, pp.92-102.

Brathwaite Dick, O. et al., 2012. The history of dengue outbreaks in the Americas. *American Journal Of Tropical Medicine And Hygiene*, 87(4), pp.584-593.

Brown, A.W.A., 1977. Yellow fever, dengue and dengue haemorrhagic fever. In: Howe, G.M. 1977. A World Geography of Human Diseases. Michigan: Academic Press.

Burkhalter, K.L. et al., 2006. Evaluation of commercial assays for detecting west nile virus antigen. *Journal of the American Mosquito Control Association*, 22(1), pp.64-69.

Burt, F.J. et al., 2012. Chikungunya: a re-emerging virus. *Lancet*, 379(9816), pp.662-671.

Cairncross, S., Muller, R. & Zagaria, N., 2002. Dracunculiasis (guinea worm disease) and the eradication initiative. *Clinical Microbiology Reviews*, 15(2), pp.223-246.

Campbell, K.M., et al., 2013. The complex relationship between weather and dengue virus transmission in Thailand. *American Journal of Tropical Medicine & Hygiene*, 89, pp.1066–1080. doi:10.4269/ajtmh.13-0321.

Capeding, M.R. et al., 2014. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet*, 384(9951), pp.1358-1365.

Cardona-Ospina, J.A., Diaz-Quijano, F.A. & Rodríguez-Morales, A.J., 2015. Burden of chikungunya in Latin American countries: estimates of disability-adjusted life-years (DALY) lost in the 2014 epidemic. *International Journal of Infectious Diseases*, 38, pp.60-61.

Castro, M. et al., 2012. A community empowerment strategy embedded in a routine dengue vector control programme: a cluster randomised controlled trial. *Transactions Of The Royal Society Of Tropical Medicine And Hygiene*, 106(5), pp.315-321.

Centre for Reviews and Dissemination, 2009. Systematic Reviews: CRD's guidance for undertaking reviews in health care. York: University of York. Available: http://www.york.ac.uk/inst/crd/pdf/Systematic_Reviews.pdf.

Chadee, D.D., 2009. Dengue cases and Aedes aegypti indices in Trinidad, West Indies. *Acta Tropica*, 112(2), pp.174-180.

Chadee, D.D., 2013. Resting behaviour of Aedes aegypti in Trinidad: with evidence for the re-introduction of indoor residual spraying (IRS) for dengue control. *Parasites & Vectors,* 6(1), pp.1-1.

Chaintoutis, S.C. et al., 2014. Surveillance and early warning of West Nile Virus lineage 2 using backyard chickens and correlation to human neuroinvasive cases. *Zoonoses and Public Health*, 62(5), pp.344-355.

Chan, E.H. et al., 2011. Using web search query data to monitor dengue epidemics: A new model for neglected tropical disease surveillance. *PLoS Neglected Tropical Diseases*, 5(5), pp.e1206.

Chan, M. & Johansson, M.A., 2012. The incubation periods of dengue viruses. *PloS One*, 7(11), p.e50972.

Chan, Y.H., 2003. Randomised controlled trials (RCTs)-essentials. *Singapore Medical Journal,* 44(2), pp.060-063.

Chang, F.-S. et al., 2015. Re-assess vector indices threshold as an early warning tool for predicting dengue epidemic in a dengue non-endemic country. *PLoS Neglected Tropical Diseases*, 9(9), p.e0004043.

Chastel, C., 2012. Eventual role of asymptomatic cases of dengue for the introduction and spread of dengue viruses in non-endemic regions. *Frontiers in Physiology*, 70, pp.1-4.

Chattopadhyay, P. et al., 2013. Ultra low concentration deltamethrin loaded patch development and evaluation of its repellency against dengue vector Aedes (S) albopictus. *Parasites & Vectors*, 6(1), pp.284-284.

Chen, S.-C. & Hsieh, M.-H., 2012. Modeling the transmission dynamics of dengue fever: Implications of temperature effects. *Science of the Total Environment*, The, 431, pp.385-391.

Chen, Y.R., Hwang, J.S. & Guo, Y.J., 1994. Ecology and control of dengue vector mosquitoes in Taiwan. *Gaoxiong Yi Xue Ke Xue Za* Zhi, S10, pp.S78-87.

Christensen, B.M., LaFond, M.M. & Christensen, L.A., 1986. Defense reactions of mosquitoes to filarial worms: effect of host age on the immune response to dirofilaria immitis microfilariae. *The Journal of Parasitology*, 72(2), pp.212-215.

Christiansen-Jucht, C.L. et al., 2014. Temperature during larval development and adult maintenance influences the survival of Anopheles gambiae s.s. *Parasites & Vectors,* 7(489), pp.1-10.

Chunara, R., Andrews, J.R. & Brownstein, J.S., 2012. Social and news media enable estimation of epidemiological patterns early in the 2010 Haitian cholera outbreak. *American Journal Of Tropical Medicine And Hygiene*, 86(1), pp.39-45.

Clark, G.G., Seda, H. & Gubler, D.J., 1994. Use of the “CDC backpack aspirator” for surveillance of Aedes aegypti in San Juan, Puerto Rico. J*ournal of the American Mosquito Control Association,* 10(1), pp.119-124.

Clements, A.N., 1999. The Biology of Mosquitoes: Sensory reception and behaviour, volume 2. New York: CABI Publishing.

Connor, M.E. & Monroe, W.M., 1923. Stegomyia indices and their value in yellow fever control. *American Journal Of Tropical Medicine*, S1-3(1), pp.9-19.

Cornet, M. et al., 1968. An epidemic of yellow fever in Senegal in 1965. Epidemiological studies. Bulletin of the World Health Organisation, 39(6), pp.845.

Cullen, J.R. et al., 1984. An epidemiological early warning system for malaria control in northern Thailand. *Bulletin of the World Health Organisation*, 62(1), pp.107-114.

Da Costa, V.G. et al., 2014. Safety, immunogenicity and efficacy of a recombinant tetravalent dengue vaccine: A meta-analysis of randomized trials. *Vaccine*, 32(39), pp.4885-4892.

Da Silva Paixão, K. et al., 2015. Volatile semiochemical-conditioned attraction of the male yellow fever mosquito, Aedes aegypti, to human hosts. *Journal of Vector Ecology*, 40(1), pp.1-6.

Dantés, H.G., Farfan-Ale, J.A. & Sarti, E., 2014. Epidemiological trends of dengue disease in Mexico (2000–2011): A systematic literature search and analysis. *PLoS Neglected Tropical Diseases*, 8(11), pp.e3158.

De Figueiredo, R.M.P. et al., 2013. Identification of dengue viruses in naturally infected Aedes aegypti females captured with BioGents (BG) Sentinel traps in Manaus, Amazonas, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, *46*, pp.221-222. http://dx.doi.org/10.1590/0037-8682-1096-2013.

Degener, C.M. et al 2014. Evaluation of mass trapping for dengue vector control - A cluster RCT in Brazil 2014. *Journal of Medical Entomology,* 51(2), pp.408-420.

De Melo, D.P.O., Scherrer, L.R. & Eiras, A.E., 2012. Dengue fever occurrence and vector detection by larval survey, ovitrap and mosquiTRAP: A space-time clusters analysis. *PLoS One*, 7(7), pp.e42125.

Demicheli, R.A, et al., 2014. Vaccines for measles, mumps and rubella in children. *Cochrane Database of Systematic Reviews*, 15(2), pp.1-163.

Doke, P.P. et al., 2000. Impact of single round of indoor residual spray with lambda-cyhalotrin 10% WP on Plasmodium falciparum infection in Akola district, Maharashtra State. *Journal of Communicable Diseases*, 32(3), pp.190-200.

Dorigatti, I. et al., 2015. Modelling the immunological response to a tetravalent dengue vaccine from multiple phase-2 trials in Latin America and South East Asia. *Vaccine*, 33(31), pp. 3746-51.

Douglas, D.L. et al., 2013. Will dengue vaccines be used in the public sector and if so, how? Findings from an 8-country survey of policymakers and opinion leaders. *PLoS Neglected Tropical Diseases*, 7(3), pp.e2127.

Duncombe, J. et al., 2013. Spatiotemporal patterns of Aedes aegypti populations in Cairns, Australia: assessing drivers of dengue transmission. *Tropical Medicine and International Health*, 18, pp.839-849. doi:10.1111/tmi.12115.

Egger, M., Smith, G.D. & Phillips, A.N., 1997. Meta-analysis: principles and procedures. *British Medical Journal*, 315(7121), pp.1533-1537.

Eiras, A.E. & Resende, M.C., 2009. Preliminary evaluation of the 'Dengue-MI' technology for Aedes aegypti monitoring and control. *Cadernos de Saude Publica*, 25(S1), 45-58. doi: 10.1590/S0102-311X2009001300005.

Eisen, L., et al., 2009. Proactive vector control strategies and improved monitoring and evaluation practices for dengue prevention. *Journal of Medical Entomology*, 46(6), pp.1245-1255.

Ellis, A.M. et al., 2011. Parameterization and sensitivity analysis of a complex simulation model for mosquito population dynamics, dengue transmission, and their control. *American Journal Of Tropical Medicine And Hygiene*, 85(2), pp.257-264.

Endy, T.P., 2002. Epidemiology of inapparent and symptomatic acute dengue virus infection: A prospective study of primary school children in Kamphaeng Phet, Thailand. *American Journal Of Epidemiology*, 156(1), pp.40-51.

Erlanger, T.E., Keiser, J. & Utzinger, J., 2008. Effect of dengue vector control interventions on entomological parameters in developing countries: a systematic review and meta-analysis. *Medical and Veterinary Entomology*, 22(3), pp.203-221.

Esu, E. et al., 2010. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Tropical Medicine & International Health*, 15(5), pp.619-631.

Frank, C. et al., 2013. More reasons to dread rain on vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012. *Eurosurveillance,* 18(14), pp.14-17.

Fan, Y. et al., 2014. Estimating the effectiveness of early control measures through school absenteeism surveillance in observed outbreaks at rural schools in Hubei, China. *PLoS One*, 9(9), p.e106856.

Farajollahi, A. et al., 2012. Effectiveness of ultra-low volume nighttime applications of an adulticide against diurnal Aedes albopictus, a critical vector of dengue and chikungunya viruses. *PLoS One*, 7(11), p.e49181.

Farrington, P. & Andrews, N., 2003. Outbreak detection: application to infectious disease surveillance. In: Brookmeyer, Ron and Stroup, Donna F. eds. Monitoring the Health of Populations: Statistical Principles and Methods for Public Health Surveillance. New York, NY, USA: OUP USA, pp. 203–231.

Favier, C., Dégallier, N. & Dubois, M.A., 2005. Dengue epidemic modeling: stakes and pitfalls. *Asia Pacific Biotech News*, 9(22), pp.1191-1194.

Fay, R.W. & Eliason, D.A., 1966. A preferred oviposition site as a surveillance method for Aedes aegypti. *Mosquitoes News*, 26, pp. 531-535.

Ferguson, H.M. et al. 2010. Ecology: a prerequisite for malaria elimination and eradication. *PLoS Medicine*, 7(8), pp.e1000303. doi: 10.1371/journal.pmed.1000303.

Focks, D. et al., 2001. Transmission thresholds for dengue in terms of Aedes aegypti pupae per person with discussion of their utility in source reduction efforts. *American Journal of Tropical Medicine & Hygiene*, 62, pp.11–18.

Focks, D. & Chadee, D.D., 1997. Pupal survey: an epidemiologically significant surveillance method for Aedes aegypti: an example using data from Trinidad. *American Journal of Tropical Medicine & Hygiene*, 56, pp.159-167. Available: http://www.ncbi.nlm.nih.gov/pubmed/9080874.

Focks, D.A., et al., 1995. A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation, and samples of simulation results. *American Journal of Tropical Medicine & Hygiene*, 53, pp.489-506.

Focks, D. & Chadee, D.D., 1997. Pupal survey: an epidemiologically significant surveillance method for Aedes aegypti: an example using data from Trinidad. *American Journal Of Tropical Medicine And Hygiene*, 56(2), pp.159-167.

Focks, D., 2004. A review of entomological sampling methods and indicators for Dengue Vectors. *World Health Organisation,* pp.1-40.

Focks, D., & Alexander, N., 2006. Multicountry study of Aedes aegypti pupal productivity survey methodology: findings and recommendations. Geneva: World Health Organisation, Special Programme for Research and Training in Tropical Diseases.

Focks, D., Kloter, K. & Carmichael, G., 1987. The impact of sequential ultra-low volume ground aerosol applications of malathion on the population dynamics of Aedes aegypti (L.). *American Journal Of Tropical Medicine And Hygiene*, 36(3), p.639.

Garcıa-Rejon, J. et al., 2008. Dengue virus-infected Aedes aegypti in the home environment. *American Journal of Tropical Medicine & Hygiene*, 79(6), pp.940-50.

Lorono-Pino, M.A., et al., 2008. Dengue virus-infected Aedes aegypti in the home environment. *American Journal of Tropical Medicine & Hygiene*, 79: 940-950. Available: http://www.ajtmh.org/content/79/6/940.long.

Gigliogi, G., 1948. An investigation of the house-frequenting habits of mosquitoes of the British Guiana coastland in relation to the use of DDT. *American Journal Of Tropical Medicine & Hygiene*, 28(1), pp.43-70.

Gluskin, R.T. et al., 2014. Evaluation of internet-based dengue query data: Google dengue trends. *PLoS Neglected Tropical Diseases*, 8(2), pp.e2713.

Godfray, H.C., 2013. Mosquito ecology and control of malaria. *Journal of Animal Ecolology*, 82(1), pp.15-25. doi: 10.1111/1365-2656.12003.

Goh, K.T., 1997 Dengue--a re-emerging infectious disease in Singapore. *Annals Academy of Medicine*, 26(5), pp.664-670.

Goh, K.T. et al., 1987. Epidemiological aspects of an outbreak of dengue fever/dengue haemorrhagic fever in Singapore. *Southeast Asian Journal of Tropical Medicine & Public Health*, 18: 295–302. Available: http://www.ncbi.nlm.nih.gov/pubmed/3433161.

Golding, N. et al., 2015. Integrating vector control across diseases. *BMC Medicine*, 13(1), pp.249.

Gouteux, J. & Sinda, D., 1990. Community participation in the control of tsetse flies. Large scale trials using the pyramid trap in the Congo. *Tropical Medicine & Parasitology*, 41(1), pp. 49-55.

Grisales, N. et al., 2013. Temephos resistance in Aedes aegypti in Colombia compromises dengue vector control. *PLoS Neglected Tropical Diseases*, 7(9), pp.e2438.

Gubler, D.J. & Clark, G.G., 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging Infectious Diseases*, 1(2), pp.55-57.

Gubler, D.J., 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Microbiology*, 10(2), pp.100-103.

Gubler, D.J., 2011. Dengue, urbanization and globalization: The unholy trinity of the 21st Century. *Tropical Medicine and Health*, 39, pp.S3-S11.

Gubler, D.J., 2012. The economic burden of dengue. *American Journal Of Tropical Medicine And Hygiene*, 86(5), pp.743-744.

Gurtler, R.E., Garelli, F.M. & Coto, H.D., 2009. Effects of a five-year citywide intervention program to control Aedes aegypti and prevent dengue outbreaks in northern Argentina. PLoS neglected tropical diseases, 3(4), p.e427. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed9&AN=2009223964.

Guzman, M.G. et al., 2002. Effect of age on outcome of secondary dengue 2 infections. *International Journal of Infectious Diseases*, 6(2), pp.118-124.

Guzman, M.G. et al., 2010. Dengue: a continuing global threat. *Nature Publishing Group*, 8(12), pp.S7-S16.

Halasa, Y.A., Shepard, D.S. & Zeng, W., 2012. Economic cost of dengue in Puerto Rico. *American Journal Of Tropical Medicine And Hygiene*, 86(5), pp.745-752.

Halide, H. & Ridd, P., 2008. A predictive model for Dengue Hemorrhagic Fever epidemics. *International Journal of Environmental Health Research*, 18(4), pp.253-265.

Halstead, S.B., 2012. Dengue vaccine development: a 75% solution? *Lancet*, 380(9853), pp.1535-6.

Han, W.W. et al., 2015. Efficacy and community effectiveness of larvivorous fish for dengue vector control. *Tropical Medicine & International Health*, 20(9), pp.1239-1256.

Hanna, J.N. et al., 2001. An epidemic of dengue 3 in far north Queensland, 1997-1999. *Medical Journal of Australia*, 174(4), pp.178-182.

Harrington, J. et al., 2013. Detecting and responding to a dengue outbreak: Evaluation of existing strategies in country outbreak response planning. *Journal of Tropical Medicine*, 2013(5), pp.1-9.

Harrington, L.C. et al., 2005. Dispersal of the dengue vector Aedes aegypti within and between rural communities. *American Journal Of Tropical Medicine And Hygiene*, 72(2), pp.209-220.

Harrington, L.C. et al., 2008. Influence of container size, location, and time of day on oviposition patterns of the dengue vector, Aedes aegypti, in Thailand. *Vector-Borne and Zoonotic Diseases*, 8(3), pp.415-424.

Harris, A.F. et al., 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology*, 30(9), pp.828-830.

Harrison, B.A. et al., 1982. An efficient floating larval trap for sampling Aedes aegypti populations (Diptera: Culicidae). *Journal of Medical Entomology,* 19(6), pp.722-727.

Harwood, J.F. et al., 2015. Assessing carbon dioxide and synthetic lure-baited traps for dengue and chikungunya vector surveillance. *Journal of the American Mosquito Control Association*, 31(3), pp.242-247.

Hati, A.K. et al., 1989. Annual transmission potential of bancroftian filariasis in an urban and a rural area of West Bengal, India. *American Journal Of Tropical Medicine And Hygiene*, 40(4), pp.365-367.

Hemingway, J. & Ranson, H., 2000. Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, 45:371–391

Hemingway, J. et al., 2006. The innovative vector control consortium: improved control of mosquito-borne diseases. *Trends in Parasitology*, 22(7), pp.308-312.

Hemingway, P. & Brereton, N., 2009. What is a systematic review? *What is Series, 9, pp.1111*. Available: http://www.medicine.ox.ac.uk/bandolier/painres/download/whatis/syst-review.pdf.

Hemming, K. et al., 2011. Sample size calculations for cluster randomised controlled trials with a fixed number of clusters. *BMC Medical Research Methodology*, 11(1), pp.102.

Hernández-Ávila, J.E. et al., 2013. Nationwide, web-based, geographic information system for the integrated surveillance and control of dengue fever in Mexico. *PLoS One*, 8(8), pp.e70231.

Higa, Y., 2011. Dengue vectors and their spatial distribution. *Tropical Medicine and Health*, 39(4), pp.S17-S27.

Higgins, J.P. & Green, S. eds, 2015. Cochrane Handbook Version 5.1.0. [online] Available at: http://handbook.cochrane.org [Accessed June 19, 2015].

Hii, Y.L. et al., 2009. Climate variability and increase in intensity and magnitude of dengue incidence in Singapore. *Global Health Action*, 2, pp.1-9.

Hii, Y.L. et al., 2012a. Forecast of dengue incidence using temperature and rainfall. *PLoS Neglected Tropical Diseases*, 6(11), pp.e1908.

Hii, Y.L., et al., 2012. Optimal lead time for dengue forecast. *PLoS Neglected Tropical Diseases*, 6(10), pp.e1848.

Hoel, D.F. et al., 2015. A comparison of carbon dioxide sources for mosquito capture in centers for disease control and prevention light traps on the Florida gulf coast. *Journal of the American Mosquito Control Association*, 31(3), pp.248-257.

Hoffmann, A.A. et al., 2011. Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature*, 476(7361), pp.454-457.

Honorio, N.A. et al., 2009. Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil. *PLoS Neglected Tropical Diseases*, 3(11) pp.e545.

Horstick, O. et al., 2010. Dengue vector-control services: how do they work? A systematic literature review and country case studies. *Transactions Of The Royal Society Of Tropical Medicine And Hygiene*, 104(6), pp.379-386.

Horstick, O. et al., 2015. WHO dengue case classification 2009 and its usefulness in practice: an expert consensus in the Americas. *Pathogens and Global Health*, 109(1), pp.19-25.

Hotez, P.J. et al., 2014. The global burden of disease study 2010: Interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical Diseases*, 8(7), pp.e2865.

Hughes, H.E. et al., 2015. Emergency department syndromic surveillance providing early warning of seasonal respiratory activity in England. *Epidemiology and Infection*, pp.1–13.

Hugo, L.E. et al., 2014. Adult survivorship of the dengue mosquito Aedes aegypti varies seasonally in central Vietnam. *PLoS Neglected Tropical Diseases*, 8(2), p.e2669.

Huy, R. et al., 2010. National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control. *Bulletin of the World Health Organisation*, 88(9), pp.650-657.

Igarashi, A., 1997. Impact of dengue virus infection and its control. *FEMS Immunology & Medical Microbiology*, 18(4), pp.291-300.

Jackson, N., Waters, E. & Waters, E., 2004. The challenges of systematically reviewing public health interventions. *Journal of Public Health*, 26(3), pp.303-307.

Jaenisch, T. et al., 2013. Dengue research funded by the European Commission-scientific strategies of three European dengue research consortia. *PLoS Neglected Tropical Diseases*, 7(12), pp.e2320.

Janwan, P. et al., 2011. Growth and development of Gnathostoma spinigerum (Nematoda: Gnathostomatidae) larvae in Mesocyclops aspericornis (Cyclopoida: Cyclopidae). *Parasites & Vectors*, 4(1), p.93.

Jayasooriya, G.A., et al., 2009. Use of geographical information system (GIS) and global positioning system (GPS) for dengue and dengue haemorrhagic fever control in Sri Lanka. *Dengue Bulletin*, 33(1), pp.11-20.

Jessie, K. et al., 2004. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. *The Journal of Infectious Diseases,* 189(8), pp.1411-1418.

Johansson, M.A., Cummings, D.A.T. & Glass, G.E., 2009. Multiyear climate variability and dengue-El Niño Southern Oscillation, weather, and dengue incidence in Puerto Rico, Mexico, and Thailand: A longitudinal data analysis. *PLoS Medicine*, 6(11), p.e1000168.

Johansson, M.A., Dominici, F. & Glass, G.E., 2009. Local and global effects of climate on dengue transmission in Puerto Rico. PLoS Neglected Tropical Diseases, 3(2), p.e382. Available at: http://dx.plos.org/10.1371/journal.pntd.0000382.s001.

Jones, C.H. et al., 2014. Use and acceptance of long lasting insecticidal net screens for dengue prevention in Acapulco, Guerrero, Mexico. *BMC Public Health*, 14(1), pp.1-10.

Kalyanasundaram, M. & Mathew, N., 2006. N,N-diethyl phenylacetamide (DEPA): A safe and effective repellent for personal protection against hematophagous arthropods. *Journal of Medical Entomology*, 43(3), pp.518-525.

Karl, S. et al., 2014. A spatial simulation model for dengue virus infection in urban areas. *BMC Infectious Diseases*, 14(1), pp.1-17.

Karunaratne, S. et al., 2013. Insecticide resistance and efficacy of space spraying and larviciding in the control of dengue vectors Aedes aegypti and Aedes albopictus in Sri Lanka. *Pesticide Biochemistry Physiology*, 107(1), pp.98-105.

Katzelnick, L.C. et al., 2015. Dengue viruses cluster antigenically but not as discrete serotypes. *Science*, 349(6254), pp.1338-1343.

Kay, B. & Nam, V.S., 2005. New strategy against Aedes aegypti in Vietnam. *Lancet*, 365(9459), pp.613-617.

Kay, B.H.B. et al., 2002. Control of aedes vectors of dengue in three provinces of Vietnam by use of Mesocyclops (Copepoda) and community-based methods validated by entomologic, clinical, and serological surveillance. *American Journal Of Tropical Medicine & Hygiene*, 66(1), pp.40-48.

Kelly-Hope, L., Ranson, H. & Hemingway, J., 2008. Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. *Lancet Infectious Diseases*, 8(6), pp.387-389.

Kennedy JS. The visual responses of flying mosquitoes. *Journal of Zoology*, 109, pp.221-242.

Kim, D., Fedak, K. & Kramer, R., 2012. Reduction of malaria prevalence by indoor residual spraying: A meta-regression analysis. *American Journal Of Tropical Medicine And Hygiene*, 87(1), pp.117-124.

Kirby, M.J. et al., 2009. Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. *Lancet*, 374(9694), pp.998-1009.

Kirby, M.J. et al., 2010. Social acceptability and durability of two different house screening interventions against exposure to malaria vectors, plasmodium falciparum infection, and anaemia in children in The Gambia, West Africa. *American Journal Of Tropical Medicine & Hygiene*, 83(5), pp.965-972.

Ko, Y.C., Chen, M.J. & Yeh, S.M., 1992. The predisposing and protective factors against dengue virus transmission by mosquito vector. *American Journal Of Epidemiology*, 136(2), pp.214-220.

Koh, B.K.W. et al., 2008. The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. *Annals Academy of Medicine Singapore*, 37, pp.538-545.

Koou, S.Y. et al., 2014. Insecticide resistance and its underlying mechanisms in field populations of Aedes aegypti adults (Diptera: Culicidae) in Singapore. *Parasites & Vectors*, 7, pp.1-15.

Kovacic, V. et al., 2013. Community acceptance of tsetse control baits: A qualitative study in Arua district, North West Uganda. *PLoS Neglected Tropical Diseases,* 7, pp.e2579.

Kroeger, A. et al., 2006. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. *British Medical Journal*, 332: 1247-1252.

Kroeger, A. & Nathan, M.B., 2006. Dengue: setting the global research agenda. *Lancet*, 368(9554), pp.2193-2195.

Kroeger, A. et al., 2014 Effective, affordable and evidence based dengue early warning and response systems: Towards a validated contingency plan. Meeting Report 22-24 April. Geneva: World Health Organisation.

Kuehn, B.M., 2014. Chikungunya virus transmission found in the United States: US health authorities brace for wider spread. *Journal of the American Medical Association*, 312(8), pp.776-777.

Kuhn, K. et al., 2005. Using climate to predict infectious disease epidemics. Geneva: World Health Organisation.

Kumar, S., 2014. Larvicidal, repellent, and irritant potential of the seed-derived essential oil of Apium graveolens against dengue vector, Aedes aegypti L. (Diptera: Culicidae). *Frontiers in Public Health*, 18(2), pp.1-6.

Kuno, G.G., 1995. Review of the factors modulating dengue transmission. *Epidemiology Reviews*, 17(2), pp.321-335.

Kusumawathie, P.H.D. et al., 2009. Effectiveness of net covers on water storage tanks for the control of dengue vectors in Sri Lanka. *Journal of Vector Borne Diseases*, 46(2), pp.160-163.

L'Azou, M. et al., 2014. Reviewing the literature for epidemiological trends of dengue disease: Introduction to a series of seven national systematic literature reviews. *PLoS Neglected Tropical Diseases*, 8(11), pp.e3260.

Lam, S.K. et al., 2011. Preparing for introduction of a dengue vaccine: Recommendations from the 1st dengue v2V Asia-Pacific meeting. *Vaccine*, 29(51), pp.9417-9422.

Lambrechts, L. et al., 2015. Assessing the epidemiological effect of wolbachia for dengue control. *Lancet Infectious Diseases*, 15(7), pp.862-866.

Lambrechts, L., Scott, T.W. & Gubler, D.J., 2010. Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. *PLoS Neglected Tropical Diseases*, 4(5), pp.e646.

Larsen, D.A. et al., 2014. Community coverage with insecticide-treated mosquito nets and observed associations with all-cause child mortality and malaria parasite infections. *American Journal Of Tropical Medicine And Hygiene*, 91(5), pp.950-958.

Lawpoolsri, S. et al., 2014. Real-time monitoring of school absenteeism to enhance disease surveillance: A pilot study of a mobile electronic reporting system. *JMIR mHealth and uHealth*, 2(2), pp.e22.

Lazaro, A. et al., 2015. Community effectiveness of copepods for dengue vector control: systematic review. *Tropical Medicine & International Health*, 20(6), pp.685-706.

Lehane, M.J. & Aksoy, S., 2012. Control using genetically modified insects poses problems for regulators. *PLoS Neglected Tropical Diseases*, 6(1), pp.e1495.

Leite, A. et al., 2014. Asymptomatic individuals and dengue transmission–insights from mathematical modeling, *European Journal of Public Health*, 24(S2) pp.1. Doi: 10.1093/eurpub/cku151.020.

Lengeler, C., 2004. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database of Systematic Reviews*, 2, pp.1-55.

Lenhart, A. et al., 2013. A cluster-randomized trial of insecticide-treated curtains for dengue vector control in Thailand. *American Journal Of Tropical Medicine & Hygiene,* 88(2), pp.254-259.

Li, Y. et al., 2014. Urbanization Increases Aedes albopictus larval habitats and accelerates mosquito development and survivorship. *PLoS Neglected Tropical Diseases*, 8(11), p.e3301.

Lima, E.P., Goulart, M.O.F. & Neto, M.L.R., 2015. Meta-analysis of studies on chemical, physical and biological agents in the control of Aedes aegypti. *BMC Public Health*, 15, pp.1-14.

Lin, T.H., 1994. Surveillance and control of Aedes aegypti in epidemic areas of Taiwan. *Gaoxiong Yi Xue Ke Xue Za Zhi*, 10, pp.S88-93.

Lindsay, S.W., Emerson, P.M. & Charlwood, J.D., 2002. Reducing malaria by mosquito-proofing houses. *Trends in Parasitology*, 18(11), pp.510-514.

Litchfield, J. et al., 2005. Is the future for clinical trials internet-based? A cluster randomized clinical trial. *Clinical Trials*, 2(1), pp.72-79.

Liu-Helmersson, J. et al., 2014. Vectorial capacity of Aedes aegypti: effects of temperature and implications for global dengue epidemic potential. *PLoS One*, 9(3), pp.e89783.

Lloyd, L.S. et al., 1994. The design of a community-based health education intervention for the control of Aedes aegypti. *American Journal Of Tropical Medicine & Hygiene*, 50(4), pp.401-411.

Loke, S.R. et al., 2015. Evaluation of sumithion L-40 against Aedes aegypti (L.) and Aedes albopictus skuse. *Tropical Biomedicine*, 32(1), 76-83.

Loroño-Pino, M.A. et al., 2013. Towards a Casa Segura: a consumer product study of the effect of insecticide-treated curtains on Aedes aegypti and dengue virus infections in the home. *American Journal Of Tropical Medicine And Hygiene*, 89(2), pp.385-397.

Louis, V.R.R. et al., 2014. Modeling tools for dengue risk mapping - a systematic review. *International Journal of Health Geography*, 13(1), pp.1-15.

Lowe, D.R. et al., 2014. Dengue outlook for the World Cup in Brazil: an early warning model framework driven by real-time seasonal climate forecasts. *Lancet Infectious Diseases*, 14(7), pp.619-626.

Luz, P.M. et al., 2011. Dengue vector control strategies in an urban setting: an economic modelling assessment. *Lancet,* 377, pp.1673-1680. Available: http://www.sciencedirect.com/science/article/pii/S0140673611602468.

Luz, P.M. et al., 2011. Dengue vector control strategies in an urban setting: an economic modelling assessment. *Lancet*, 377(9778), pp.1673-1680. Available at: http://www.sciencedirect.com/science/article/pii/S0140673611602468.

MacDonald, G., 1952. The analysis of the sporozoite rate. *Tropical Diseases Bulletin*, 49(6), pp.569–586.

MacDonald, G., 1957. The epidemiology and control of malaria. London: Oxford University Press.

Macdonald, W.W., 1956. Aedes aegypti in Malaya. II. Larval and adult biology. *Annals of Tropical Medicine and Parasitology*, (56), pp.399-414.

Maciel-de-Freitas, R. et al., 2008. Mosquito traps designed to capture Aedes aegypti (Diptera: Culicidae) females: preliminary comparison of Adultrap, MosquiTRAP and backpack aspirator efficiency in a dengue-endemic area of Brazil. *Memorio Instituto Oswaldo Cruz*, 103, pp.602-5.

Maciel-de-Freitas, R. & Lourenço-de-Oliveira, R., 2011. Does targeting key-containers effectively reduce Aedes aegypti population density? *Tropical Medicine & International Health*, 16, pp.965-973.

Maciel-de-Freitas, R. & Valle, D., 2014. Challenges encountered using standard vector control measures for dengue in Boa Vista, Brazil. *Bulletin of the World Health Organisation*, 92(9), pp.685-689.

Maciel-de-Freitas, R. et al., 2007. Variation in Aedes aegypti (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. *Memórias do Instituto Oswaldo Cruz*, 102(4), pp.489-496.

Maciel-de-Freitas, R. et al., 2012. Why do we need alternative tools to control mosquito-borne diseases in Latin America? Memórias do Instituto Oswaldo Cruz, 107(6), pp.828–829.

Maciel-De-Freitas, R., David, M.R. & Ferreira, A.A., 2009. Targeting productive container and positive houses as control strategies to reduce Aedes aegypti population density in dengue endemic neighborhoods. *American Journal Of Tropical Medicine And Hygiene*, 1, pp.70.

Madoff, L.C., Fisman, D.N. & Kass-Hout, T., 2011. A new approach to monitoring dengue activity. *PLoS Neglected Tropical Diseases*, 5(5), pp.e1215.

Maia, M.F. et al., 2011. Comparison of the CDC backpack aspirator and the Prokopack aspirator for sampling indoor- and outdoor-resting mosquitoes in southern Tanzania. *Parasites & Vectors*, 4(1), pp.124.

Mammen, M.P. et al., 2008. Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Medicine*, 5(11), pp.1605–1616.

Manda, H. et al., 2013. Contact irritant responses of Aedes aegypti using sublethal concentration and focal application of pyrethroid chemicals. *PLoS Neglected Tropical Diseases*, 7(2), pp.e2074.

Manore, C.A. et al., 2014. Towards an early warning system for forecasting human West Nile Virus incidence. *PLoS Current Outbreaks*, 6, pp.1-20.

Manrique-Saide, P. et al., 2008. Pupal surveys for Aedes aegypti surveillance and potential targeted control in residential areas of Mérida, México. *Journal of the American Mosquito Control Association*, 24(2), pp.289-298.

Manrique-Saide, P. et al., 2011. Operational guide for assessing the productivity of Aedes aegypti breeding sites. Geneva: World Health Organisation.

Manrique-Saide, P. et al., 2015. Use of Insecticide-Treated House Screens to Reduce Infestations of Dengue Virus Vectors, Mexico. *Emerging Infectious Diseases*, 21(2), pp.308-11.

Marcombe, S. et al., 2011. Pyrethroid resistance reduces the efficacy of space sprays for dengue control on the island of Martinique (Caribbean). *PLoS Neglected Tropical Diseases*, 5(6), pp.e1202.

Marcombe, S., Darriet, F., Agnew, P., et al., 2011. Field efficacy of new larvicide products for control of multi-resistant Aedes aegypti populations in Martinique (French West Indies). *American Journal Of Tropical Medicine And Hygiene*, 84(1), pp.118-126.

Marcombe, S. et al., 2011. Resistance of Aedes aegypti to insecticides in Martinique and implications for dengue vector control. *Tropical Medicine & International Health*, 16, pp.284.

Martelli, C.M.T. et al. (2011) Quality of life among adults with confirmed dengue in Brazil. *American Journal of Tropical Medicine & Hygiene*, 85, pp.732-738. doi:10.4269/ajtmh.2011.11-0067.

May, S. & Bigelow, C., 2005. Modeling nonlinear dose-response relationships in epidemiologic studies: Statistical approaches and practical challenges. *Dose-Response*, 3(4), pp.474-490.

McBride, C.S. et al., 2014. Evolution of mosquito preference for humans linked to an odorant receptor. *Nature*, 515(7526), pp.222-227.

McBride, W.J. et al., 1998. Determinants of dengue 2 infection among residents of Charters Towers, Queensland, Australia. *American Journal Of Epidemiology*, 148(11), pp.1111-1116.

McCall, P.J. & Kittayapong, P., 2007. Control of dengue vectors: tools and strategies. Report of the Scientific Working Group on Dengue. Geneva, World Health Organisation.

McNaughton, D. & Duong, T.T.H., 2014. Designing a community engagement framework for a new dengue control method: A case study from central Vietnam. *PLoS Neglected Tropical Diseases*, 8(5), pp.e2794.

Messenger, A.M. et al., 2014. Serological evidence of ongoing transmission of dengue virus in permanent residents of Key West, Florida. *Vector-Borne and Zoonotic Diseases*, 14(11), pp.783-787.

Messina, J.P. et al., 2014. Global spread of dengue virus types: mapping the 70 year history. *Trends in Microbiology*, 22(3), pp.138-146.

Meza-Ballesta, A. & Gonima, L., 2014. The influence of climate and vegetation cover on the occurrence of dengue cases (2001-2010). *Revista Salud Publica*, 16(2), pp.293-306.

Mia, M.S. et al., 2013. Trends of dengue infections in Malaysia, 2000-2010. *Asian Pacific Journal of Tropical Medicine*, 6(6), pp.462-466.

Midega, J. et al., 2006. Application of the pupal/demographic-survey methodology to identify the key container habitats of Aedes aegypti (L.) in Malindi district, Kenya. *Annals of Tropical Medicine and Parasitology*, 100, pp.S61-S72.

Miller, T.A., 2011. Let high-tech genetically modified insects counter dengue. *BioScience*, 61(8), pp.586-587.

Moher, D. et al., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *Open Medicine*, 3(3), pp.e123-e130.

Moreira, L.A. et al., 2009. A wolbachia symbiont in Aedes aegypti limits infection with dengue, chikungunya, and plasmodium. *Cell*, 139(7), pp.1268-1278.

Morens, D.M. et al., 1986. Dengue in Puerto Rico, 1977: public health response to characterize and control an epidemic of multiple serotypes. *American Journal Of Tropical Medicine And Hygiene,* 35(1), pp.197-211.

Morgan, C. et al., 2015. Nosocomial transmission of dengue fever via needlestick. An occupational risk. *Travel Medicine and Infectious Disease*, 13(3), pp.271-273.

Morin, C.W., Comrie, A.C. & Ernst, K., 2013. Climate and dengue transmission: evidence and implications. *Environmental Health Perspectives*, 121(11-12), pp.1264-1272.

Morrison, A.C. et al., 2004. Temporal and geographic patterns of Aedes aegypti (Diptera: Culicidae) production in Iquitos, Peru. *Journal of Medical Entomology*, 41, pp.1123-42.

Morrison AC, Sihuincha M, Stancil JD, Zamora E, Astete H, et al. (2006) Aedes aegypti (Diptera: Culicidae) production from non-residential sites in the Amazonian city of Iquitos, Peru. *Annals of Tropical Medical Parasitology*, 100(S1), pp.S73-S86. doi:10.1179/136485906X105534.

Morrison, A.C. et al., 2008. Defining challenges and proposing solutions for control of the virus vector Aedes aegypti. *PLoS Medicine*, 5(3), pp.e68.

Morrison, A.C. et al., 2010. Epidemiology of dengue virus in Iquitos, Peru 1999 to 2005: Interepidemic and epidemic patterns of transmission. *PLoS Neglected Tropical Diseases*, 4(5), pp.e670.

Medical Research Council, 2000. A framework for development and evaluation of RCTs for complex interventions to improve health. *Medical Research Council*, pp.1-19.

Muller, D.A. et al., 2012. A portable approach for the surveillance of dengue virus-infected mosquitoes. *Journal of Virological Methods,* 183(1), pp.90-93.

Mulrow, C.D., 1994. Systematic Reviews: Rationale for systematic reviews. *British Medical Journal*, 309(6954), pp.597-599.

Murray-Smith, S., Weinstein, P. & Skelly, C., 1996. Field epidemiology of an outbreak of dengue fever in Charters Towers, Queensland: are insect screens protective? *Australian & New Zealand Journal of Public Health*, 20(5), pp.545-547.

Musso, D., Van Mai Cao-Lormeau & Gubler, D.J., 2015. Correspondence. The Lancet, 386(9990), pp.243-244.

N'Guessan, R. et al., 2010. Control of pyrethroid and DDT-resistant Anopheles gambiae by application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl. *Malaria Journal*, 9, pp.44.

Naish, S. et al., 2014. Climate change and dengue: a critical and systematic review of quantitative modelling approaches. *BMC Infectious Diseases*, 14(1), pp.1-14.

Nam, V.S. et al., 2012. Community-based control of Aedes aegypti by using mesocyclops in southern Vietnam. *American Journal Of Tropical Medicine & Hygiene*, 86(5), pp.850-859.

Nathan, M.B. & Giglioli, M.E., 1982. Eradication of Aedes aegypti on Cayman Brac and Little Cayman, West Indies, with abate (temephos) in 1970-1971. *Bulletin of the Pan American Health Organization*, 16(1), pp.28-39.

Nathan, M.B., Focks, D. & Kroeger, A., 2006. Pupal/demographic surveys to inform dengue-vector control. *Annals of Tropical Medicine and Parasitology*, 100 S1(3), pp.S1-S3.

National Collaborating Centre for Methods and Tools (2008) Quality assessment tool for quantitative studies. Hamilton, ON: McMaster University. (Updated 13 April, 2010). Retrieved from http://www.nccmt.ca/registry/view/eng/14.html.

Nayar, J.K. & Sauerman, D.M., 1973. A comparative study of flight performance and fuel utilization as a function of age in females of Florida mosquitoes. *Journal of Insect Physiology*, 19(10), pp.1977-1988.

Ndiath, M.O. et al., 2014. Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to Plasmodium falciparum: a hindrance for vector control. *Malaria Journal*, 13, pp.340.

Ngoagouni, C., 2015. Invasion of Aedes albopictus (Diptera: Culicidae) into central Africa: what consequences for emerging diseases? *Parasites & Vectors*, 8, pp.191.

Nguyen, H.T. et al., 2009. Evaluation of bifenthrin applications in tires to prevent Aedes mosquito breeding. *Journal of the American Mosquito Control Association*, 25(1), pp.74-82.

Ninphanomchai, S. et al., 2014. Predictiveness of disease risk in a global outreach tourist setting in Thailand using meteorological data and vector-borne disease incidences. *International Journal of Environmental Research and Public Health*, 11(10), pp.10694-10709.

Nisalak, A. et al., 2003. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *American Journal Of Tropical Medicine And Hygiene*, 68(2), pp.191-202.

Ocampo, C.B. et al., 2014. Reduction in dengue cases observed during mass control of Aedes (Stegomyia) in street catch basins in an endemic urban area in Colombia. *Acta Tropica*, 132, pp.15–22.

Ogoma, S.B. et al., 2012. Spatial repellency of transfluthrin-treated hessian strips against laboratory-reared Anopheles arabiensis mosquitoes in a semi-field tunnel cage. *Parasites & Vectors*, 5(1), pp.54.

Oh, H.B. et al., 2015. Bitten by a bug or a bag? Transfusion-transmitted dengue: a rare complication in the bleeding surgical patient. Transfusion, 55(7), pp.1655-1661.

Oléron Evans, T.P. & Bishop, S.R., 2014. A spatial model with pulsed releases to compare strategies for the sterile insect technique applied to the mosquito Aedes aegypti. *Mathematical Biosciences*, 254, pp.6-27.

Omar, M. et al., 2011. Field evaluation on the effectiveness of a modified approach of chemical fogging against the conventional fogging in controlling dengue outbreak. *Malaysian Journal of Pathology*, 33(2), pp.113-117.

Osorio, D.P.J.E. et al., 2014. Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study. *Lancet Infectious Diseases*, 14(9), pp.830-838.

Overgaard, H., 2006. Malaria mosquito resistance to agricultural insecticides: Risk area mapping in Thailand. *International Water Management Institute*, 103, pp.1-68.

Pai, H.H., Hong, Y.J. & Hsu, E.L., 2006. Impact of a short-term community-based cleanliness campaign on the sources of dengue vectors: An entomological and human behavior study. *Journal of Environmental Health*, 68(6), pp.35-39.

Paixão, E.S. et al., 2015. Trends and factors associated with dengue mortality and fatality in Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 48(4), pp.399-405.

Pal, S. et al., 2015. Detection of dengue virus in mosquito extracts and human clinical samples using a field expedient molecular platform. *Military Medicine*, 180(9), pp.937-942.

Paul, R. et al., 2014. Mosquito control might not bolster imperfect dengue vaccines. *Lancet*, 384(9956), pp.1747-1748.

Paz-Soldan, V.A. et al., 2011. Initial assessment of the acceptability of a push-pull Aedes aegypti control strategy in Iquitos, Peru and Kanchanaburi, Thailand. *American Journal Of Tropical Medicine & Hygiene*, 84(2), pp.208–217.

Pepin, K.M. et al., 2013. Cost-effectiveness of novel system of mosquito surveillance and control, Brazil. *Emerging Infectious Diseases*, 19(4), pp.542. doi 10.3201/eid1904.120117.

Perich, M.J. et al., 2000. Behavior of Resting Aedes aegypti (Culicidae: Diptera) and Its Relation to Ultra-low Volume Adulticide Efficacy in Panama City, Panama. Journal of medical entomology, 37(4), pp.541-546.

Perich, M.J. et al., 2003. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Medical and Veterinary Entomology*, 17(2), pp.205-210.

Pessanha, J.E.M. et al., 2009. Evaluation of the Brazilian national dengue control plan. *Cadernos de Saude Publica,* 25(7), pp.1637-41.

Pham, H.V. et al., 2011. Ecological factors associated with dengue fever in a central highlands province, Vietnam. *Stochastic Environmental Research and Risk Assessment,* 25(172), pp.485-494.

Phung, D. et al., 2015. Identification of the prediction model for dengue incidence in Can Tho city, a Mekong Delta area in Vietnam. *Acta Tropica*, 141, pp.88-96.

Picado, A. et al., 2010. Effect of village-wide use of long-lasting insecticidal nets on visceral Leishmaniasis vectors in India and Nepal: a cluster randomized trial. *PLoS Neglected Tropical Diseases*, 4(1), p.e587.

Pilger, D. et al., 2010. Dengue outbreak response: documented effective interventions and evidence gaps. *TropIKA*, 1(1), pp.1-31.

Pilger, D. et al., 2011. Is routine dengue vector surveillance in central Brazil able to accurately monitor the Aedes aegypti population? Results from a pupal productivity survey. *Tropical Medicine & International Health*, 16(9), pp.1143-1150.

Pinho, S.T. et al., 2010. Modelling the dynamics of dengue real epidemics. *Philosophical Transactions of the Royal Society A*, 368(1933), pp.5679-5693.

Pluess, B. et al., 2013. Indoor residual spraying for preventing malaria. *Cochrane Database of Systematic Reviews*, 4, pp.1-49.

Rabaa MA, Simmons CP, Fox A, Le MQ, Nguyen TTT, et al. (2013) Dengue virus in sub-tropical northern and central Viet Nam: Population immunity and climate shape patterns of viral invasion and maintenance. *PLoS Neglected Tropical Diseases*, 7(12): pp.e2581. doi:10.1371/journal.pntd.0002581.

Rabaa, M.A. et al., 2013. Dengue virus in sub-tropical northern and central Viet Nam: Population immunity and climate shape patterns of viral invasion and maintenance. *PLoS Neglected Tropical Diseases*, 7(12), pp.e2581.

Racloz, V. et al., 2012. Surveillance of dengue fever virus: A review of epidemiological models and early warning systems. *PLoS Neglected Tropical Diseases*, 6(5), pp.e1648.

Ramsey, J.M. et al., 2014. A regulatory structure for working with genetically modified mosquitoes: Lessons from Mexico. *PLoS Neglected Tropical Diseases*, 8(3), pp.e2623.

Ranson, H. et al., 2010. Insecticide resistance in dengue vectors. *TropIKA*, 1(1), pp.1-12.

Rapley, L.P. et al., 2009. A lethal ovitrap-based mass trapping scheme for dengue control in Australia: II. Impact on populations of the mosquito Aedes aegypti. *Medical and Veterinary Entomology*, 23(4), pp.303-316.

Reddy, P.B., 1977. Separation of random noise models from seasonal time series data. *Applied Mathematical Modelling*, 1(7), pp.367-371.

Reeves, R.G. et al., 2012. Scientific standards and the regulation of genetically modified insects. *PLoS Neglected Tropical Diseases*, 6(1), pp.e1502.

Reich, N.G. et al. 2013. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. *Journal of The Royal Society Interface,* 10(86), pp.1-9. Doi:10.3201/eid1302.060539.

Reid, R.D. & Sanders, N.R., 2004. Operations Management. 5th ed. United States: Wiley.

Reiner, R.C. et al., 2014. PNAS Plus: From the cover: Time-varying, serotype-specific force of infection of dengue virus. *Proceedings of the National Academy of Sciences of the United States of America*, 111(26), pp.E2694-E2702.

Renganathan, E. et al., 2003. Towards sustaining behavioural impact in dengue prevention and control. *Dengue Bulletin*, 27, pp.6-12.

Rezza, G. et al., 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet*, 370(9602), pp.1840-1846.

Rezza, G., 2014. Dengue and chikungunya: long-distance spread and outbreaks in naïve areas. *Pathogens and Global Health*, 108(8), pp.349-355.

Rigau-Pérez, J.G. et al., 1999. A deviation bar chart for detecting dengue outbreaks in Puerto Rico. *American Journal of Public Health*, 89(3), pp.374-378.

Ritchie, S.A. et al., 2014. Field validation of the gravid Aedes trap (GAT) for collection of Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology, 51, pp.210-9.

Ritchie, S.A. et al., 2013. An explosive epidemic of DENV-3 in Cairns, Australia. PLoS One 8(7), pp.e68137. doi:10.1371/journal.pone.0068137.

Ritchie, S.A. et al., 2008. A biodegradable lethal ovitrap for control of container-breeding Aedes. *Journal of the American Mosquito Control Association*, 24(1), pp.47-53.

Ritchie, S.A. et al., 2013. An explosive epidemic of DENV-3 in Cairns, Australia. PloS One, 8(7), pp.e68137.

Rodenhuis-Zybert, I.A., Wilschut, J. & Smit, J.M., 2010. Dengue virus life cycle: viral and host factors modulating infectivity. *Cellular and Molecular Life Sciences*, 67(16), pp.2773-2786.

Rodrigues, H.S. et al., 2011. Dengue disease, basic reproduction number and control. *International Journal of Computing Mathematics,* 89(3), pp.334-346.

Rodriguez, M.M., Bisset, J.A. & Fernandez, D., 2007. Levels of insecticide resistance and resistance mechanisms in Aedes aegypti from some Latin American countries. *Journal of the American Mosquito Control Association*, 23(4), pp.420-429.

Rosenbaum, L., 2015. Communicating uncertainty--Ebola, public health, and the scientific process. *New England Journal of Medicine*, 372(1), pp.7-9.

Ross, S.R., 1910. The Prevention of Malaria. London: Murray.

Rueda, L.M. et al., 1990. Temperature-dependent development and survival rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology*, 27(5), pp.892-898.

Runge-Ranzinger S, Horstick O, Marx M, Kroeger A (2008) What does dengue disease surveillance contribute to predicting and detecting outbreaks and describing trends? *Tropical Medicine & International Health*, 13: 1022-1041. doi:10.1111/j.1365-3156.2008.02112.x.

Runge-Ranzinger, S. et al., 2008. What does dengue disease surveillance contribute to predicting and detecting outbreaks and describing trends? *Tropical Medicine & International Health*, 13(8), pp.1022-1041.

Runge-Ranzinger, S. et al., 2014. Dengue disease surveillance: an updated systematic literature review. *Tropical Medicine & International Health*, 19(9), pp.1116-1160.

Russell, T.L. et al., 2014. Evaluating the feasibility of using insecticide quantification kits (IQK) for estimating cyanopyrethroid levels for indoor residual spraying in Vanuatu. *Malaria Journal*, 13(1), pp.1-8.

San Martin, J.L. et al., 2010. The epidemiology of dengue in the Americas over the last three decades: A worrisome reality. *American Journal Of Tropical Medicine & Hygiene*, 82(1), pp.128-135.

Sanchez, L. et al., 2008. A community education strategy to promote participation in dengue prevention in Cuba. *Revista Panamericana de Salud Pública,* 24(1), pp.61-69.

Sanchez, L. et al., 2009. Intersectoral coordination, community empowerment and dengue prevention: six years of controlled interventions in Playa Municipality, Havana, Cuba. *Tropical Medicine & International Health*, 14(11), pp.1356-1364.

Sanghong, R. et al., 2015. Remarkable repellency of Ligusticumsinense (Umbelliferae), a herbal alternative against laboratory populations of Anopheles minimus and Aedes aegypti (Diptera: Culicidae). *Malaria Journal*, pp.1-9.

SantAna, D.C., Sa, I.L.R. de & Sallum, M.A.M., 2014. Effectiveness of Mosquito Magnet® trap in rural areas in the southeastern tropical Atlantic Forest. *Memórias do Instituto Oswaldo Cruz*, 109(8), 1045-9.

Schaffner, F. et al., 2008. Development of Aedes albopictus risk Maps. avia-gis.com.
Schafrick NH, Milbrath MO, Berrocal VJ, Wilson ML, Eisenberg JNS (2013) Spatial clustering of Aedes aegypti related to breeding container characteristics in coastal Ecuador: Implications for dengue control. *American Journal of Tropical Medicine & Hygiene*, 89, pp.758-765.

Schoeler, G.B. et al., 2004. Evaluation of surveillance devices for monitoring Aedes aegypti in an urban area of northeastern Peru. *Journal of the American Mosquito Control Association*, 20(1), pp.6-11.

Schoof, H.F., 1967. Mating, resting habits and dispersal of Aedes aegypti. *Bulletin of the World Health Organisation*, 36(4), pp.600-601.

Scott TW, Morrison AC (2003) Aedes aegypti density and the risk of dengue virus transmission. In: Takken W, Scott TW (eds) Ecological aspects for application of genetically modified mosquitoes. Netherlands: FRONTIS. Available: http://library.wur.nl/ojs/index.php/frontis/article/view/849.

Scott, T.W. & Morrison, A.C., 2010. Vector dynamics and transmission of dengue virus: Implications for dengue surveillance and prevention strategies vector dynamics and dengue prevention. *Current Topics in Microbiology & Immunology,* 338, pp.115-128.

Selck, F.W., Adalja, A.A. & Boddie, C.R., 2014. An estimate of the global health care and lost productivity costs of dengue. *Vector-Borne and Zoonotic Diseases*, 14(11), pp.824-826.

Semenza, J., 2015. Prototype early warning systems for vector-borne diseases in Europe. *International Journal of Environmental Research and Public Health*, 12(6), pp.6333-6351.

Semenza, J.C. et al., 2014. International dispersal of dengue through air travel: Importation risk for Europe. *PLoS Neglected Tropical Diseases*, 8(12), pp.e3278.

Service, M.W., 2008. Mosquito ecology: field sampling methods. 3rd ed. Netherlands: Springer.

Shepard, D.S.D., Undurraga, E.A.E. & Halasa, Y.A.Y., 2013. Economic and disease burden of dengue in southeast Asia. *PLoS Neglected Tropical Diseases*, 7(2), pp.e2055-e2055.

Shewhart, W.A., 1931. Economic control of quality of manufactured product. New York: Van Nostrand Company Incorporated.

Shibl, A., Senok, A. & Memish, Z., 2012. Infectious diseases in the Arabian Peninsula and Egypt. *Clinical Microbiology and Infection*, 18(11), pp.1068–1080. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1198743X14607424.

Shi, Y. et al., 2015. Three-Month Real-Time Dengue Forecast Models: An Early Warning System for Outbreak Alerts and Policy Decision Support in Singapore. *Environmental Health Perspectives,* [ahead of print]. doi:10.1289/ehp.1509981.

Simmons, C.P. et al., 2012. Dengue. *New England Journal of Medicine*, 366(15), pp.1423–1432.

Sivan, A. et al., 2015. Studies on insecticide susceptibility of Aedes aegypti (Linn) and Aedes albopictus (Skuse) vectors of dengue and chikungunya in Andaman and Nicobar Islands, India. *Parasitology Research*, 114(12), pp.4693-4702.

Smith, D.L. et al., 2012. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS Pathogens*, 8(4), pp.e1002588.

Smith, C. E., 1956. The history of dengue in tropical Asia and its probable relationship to the mosquito Aedes aegypti. *Journal of Tropical Medicine & Hygiene*, 59, pp.243-251.

Sommerfeld, J. & Kroeger, A., 2012. Eco-bio-social research on dengue in Asia: a multicountry study on ecosystem and community-based approaches for the control of dengue vectors in urban and peri-urban Asia. *Pathogens and Global Health*, 106(8), pp.428-435.

Soper, F.L., 1967. Aedes aegypti and yellow fever. *Bulletin of the World Health Organisation*, 36(4), pp.521-527.

Soper, F.L., 1967. The prospects for Aedes aegypti eradication in Asia in the light of its eradication in Brazil. *Bulletin of the World Health Organisation*, 36(4), pp.645-647.

Stahl, H.-C. et al., 2013. Cost of dengue outbreaks: literature review and country case studies. *BMC Public Health*, 13(1), pp.1048-1048.

Staples, J.E. & Fischer, M., 2014. Chikungunya virus in the Americas--what a vectorborne pathogen can do. *New England Journal of Medicine*, 371(10), pp.887-889.

Stoddard, S.T. et al. 2013. House-to-house human movement drives dengue virus transmission. *Proceedings of the Natural Academy of Sciences of the United States of America*, 110(3), pp.994-9. doi: 10.1073/pnas.1213349110.

Stoddard, S.T. et al. 2009. The role of human movement in the transmission of vector-borne pathogens. *PLoS Neglected Tropical Diseases*, 3(7), pp.e481. doi:10.1371/journal.pntd.0000481.

Stoddard, S.T. et al., 2013. House-to-house human movement drives dengue virus transmission. Proceedings of the National Academy of Sciences of the United States of America, 110(3), pp.994-999.

Stoddard, S.T. et al., 2014. Long-term and seasonal dynamics of dengue in Iquitos, Peru. *PLoS Neglected Tropical Diseases*, 8(7), pp.e3003.

Strode, C. et al., 2014. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African Anopheline mosquitoes: systematic review and meta-analysis. *PLoS Medicine*, 11(3), p.e1001619.

Stroup, D.F. et al., 1989. Detection of aberrations in the occurrence of notifiable diseases surveillance data. *Statistics in Medicine*, 8(3), pp.323-9.

Styer, L.M., Carey, J.R., et al., 2007. Mosquitoes do senesce: departure from the paradigm of constant mortality. *American Journal of Tropical Medicine & Hygiene*, 76(1), pp.111-7.

Styer, L.M., Meola, M.A. & Kramer, L.D., 2007. West Nile virus infection decreases fecundity of Culex tarsalis females. *Journal of Medical Entomology*, 44(6), pp.1074-1085.

Sudre, B. et al., 2013. Mapping environmental suitability for malaria transmission, Greece. *Emerging Infectious Diseases*, 19(5), pp.1-3.

Swaddiwudhipong, W. et al., 1992. Effect of health education on community participation in control of dengue hemorrhagic fever in an urban area of Thailand. *Southeast Asian Journal of Tropical Medicine & Public Health*, 23(2), pp.200–206.

Sylvestre, G., Gandini, M. & Maciel-de-Freitas, R., 2013. Age-dependent effects of oral infection with dengue virus on Aedes aegypti (Diptera: Culicidae) feeding behavior, survival, oviposition success and fecundity. PLoS One, 8(3), pp.e59933.

Tam, C.C. et al., 2013. Estimates of dengue force of infection in children in Colombo, Sri Lanka. *PLoS Neglected Tropical Diseases*, 7(6), pp.e2259.

Tan, C.H. et al., 2011. Evaluation of the dengue NS1 Ag strip ®for detection of dengue Virus antigen in Aedes aegypti (Diptera: Culicidae). *Vector-Borne and Zoonotic Diseases*, 11(6), pp.789-792.

Tan, F.L.-S. et al., 2005. Dengue haemorrhagic fever after living donor renal transplantation. *Nephrology Dialysis Transplantation*, 20(2), pp.447-448.

Tangena, J.-A.A. et al., 2013. Alternative treatments for indoor residual spraying for malaria control in a village with pyrethroid and DDT resistant vectors in The Gambia. *PLoS One*, 8(9), p.e74351.

Taylor, F. et al., 2013. Statins for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews*, 1, pp.4816-4816.

Thai, K.T.D. & Anders, K.L., 2011. The role of climate variability and change in the transmission dynamics and geographic distribution of dengue. *Experimental Biology and Medicine*, 236(8), pp.944-954.

Thammapalo, S., Meksawi, S. & Chongsuvivatwong, V., 2012. Effectiveness of space spraying on the transmission of dengue/dengue hemorrhagic fever (DF/DHF) in an urban area of Southern Thailand. *Journal of Tropical Medicine*, 2012(1), pp.1-7.

Thomas, H. et al., 2004. A process for systematically reviewing the literature: Providing the research evidence for public health nursing interventions. *Worldviews on Evidence Based Nursing*, 1(3), pp.176-184.

Thomas, S.J. et al., 2015. Improving dengue virus capture rates in humans and vectors in Kamphaeng Phet Province, Thailand, using an enhanced spatiotemporal surveillance strategy. *American Journal Of Tropical Medicine And Hygiene*,

Thomas, S.J., 2011. The necessity and quandaries of dengue vaccine development. *Journal of Infectious Diseases*, 203(3), pp.299-303.

Toledo, M.E. et al., 2007. Towards active community participation in dengue vector control: results from action research in Santiago de Cuba, Cuba. *Transactions Of The Royal Society Of Tropical Medicine And Hygiene*, 101(1), pp.56-63.

Toledo, M.E. et al., 2011. Evidence on impact of community-based environmental management on dengue transmission in Santiago de Cuba. *Tropical Medicine & International Health*, 16(6), pp.744-747.

Tomasello, D. & Schlagenhauf, P., 2013. Chikungunya and dengue autochthonous cases in Europe, 2007-2012. *Travel Medicine and Infectious Disease*, 11(5), pp.274-284.

Tran, H.P. et al., 2010. Householder perspectives and preferences on water storage and use, with reference to dengue, in the Mekong Delta, southern Vietnam. *International Health*, 2(2), pp.136-142.

Tsunoda, T. et al., 2013. Field trial on a novel control method for the dengue vector, Aedes aegypti by the systematic use of Olyset. *Parasites & Vectors*, 6(1), pp.1-1.

Tun-Lin W, Kay BH, Barnes A, Forsyth S (1996) Critical examination of Aedes aegypti indices: correlations with abundance. *American Journal of Tropical Medicine & Hygiene*, 54, pp.543-547. Available: http://www.ncbi.nlm.nih.gov/pubmed/8644913.

Tun-Lin, W., Burkot, T.R. & Kay, B.H., 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector Aedes aegypti in north Queensland, Australia. *Medical and veterinary entomology*, 14(1), pp.31-37.

Uiskm, M., 2012. Incidence and prevalence of dengue in Bandar Lampung 2012. [Internal Report]. Bandar Lampung: Dinkas.

Undurraga, E.A. Halasa, Y.A. & Shepard, D.S., 2013. Use of expansion factors to estimate the burden of dengue in southeast Asia: A systematic analysis. PLoS Neglected Tropical Diseases, 7, pp.e2056. doi:10.1371/journal.pntd.0002056.s003.

Van Benthem, B.H.B. et al., 2005. Spatial patterns of and risk factors for seropositivity for dengue infection. *American Journal Of Tropical Medicine And Hygiene*, 72(2), pp.201-208.

Van Panhuis, W.G. et al., 2015. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. *Proceedings of the National Academy of Sciences of the United States of America*, 112(42), pp.13069-13074.

Vanlerberghe, V. et al., 2010. Community involvement in dengue vector control: Cluster randomised trial (Reprinted from British Medical Journal, vol 338, b1959, 2009). *Medic Review*, 12(1), pp.41-47.

Vanlerberghe, V. et al., 2011. Evaluation of the effectiveness of insecticide treated materials for household level dengue vector control. *PLoS Neglected Tropical Diseases*, 5(3), p.e994.

Vanlerberghe, V. et al., 2013. Coverage-dependent effect of insecticide-treated curtains for dengue control in Thailand. *American Journal Of Tropical Medicine And Hygiene*, 89(1), pp.93-98.

Vasilakis, N et al., 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nature Reviews Microbiology*, 9, pp.532-541.

Vazquez-Prokopec, G.M., 2010. Quantifying the spatial dimension of dengue virus epidemic spread within a tropical urban environment. *PLoS Neglected Tropical Diseases*, 4(12), pp.e920. doi:10.1371/journal.pntd.0000920.

Vazquez-Prokopec, G.M. et al., 2009. A new, cost-effective, battery-powered aspirator for adult mosquito collections. *Journal of Medical Entomology*, 46(6), pp.1256-1259.

Vazquez-Prokopec, G.M. et al., 2010. Quantifying the spatial dimension of dengue virus epidemic spread within a tropical urban environment. PLoS Neglected Tropical Diseases, 4(12), pp.e920.

Vazquez-Prokopec, G.M., Stoddard, S.T., et al., 2009. Usefulness of commercially available GPS data-loggers for tracking human movement and exposure to dengue virus. *International Journal of Health Geographics*, 8(1), p.68.

Verhulst, N.O. et al., 2011. Composition of human skin microbiota affects attractiveness to malaria mosquitoes. PLoS One, 6(12), pp.e28991.

Victor, T.J. et al., 2002. Dengue fever outbreaks in two villages of Dharmapuri district in Tamil Nadu. *Indian Journal of Medical Research*, 116, pp.133-139.

Voge, N.V. et al., 2013. Detection of dengue virus NS1 antigen in infected Aedes aegypti using a commercially available kit. *American Journal of Tropical Medicine and Hygiene*, 88(2), pp.260–266. Available at: http://www.ajtmh.org/cgi/doi/10.4269/ajtmh.2012.12-0477.

Vu, S.N. et al., 2005. Elimination of dengue by community programs using Mesocyclops (Copepoda) against Aedes aegypti in central Vietnam. *American Journal Of Tropical Medicine And Hygiene*, 72(1), pp.67-73.

Wagman, J.M. et al., 2015. The field evaluation of a push-pull system to control malaria vectors in Northern Belize, Central America. pp.1-11.

Walker, T. et al., 2014. The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. *Nature*, 476(7361), pp.450-453.

Walshe, D.P. et al., 2013. Larvivorous fish for preventing malaria transmission. Cochrane Database of Systematic Reviews, 12, pp.CD008090–CD008090.

Wang, E. et al., 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *Journal of Virology*, 74(7), pp.3227-3234.

Wang, N.C., 1994. Control of Dengue Vectors in Singapore. *Gaoxiong Yi Xue Ke Xue Za Zhi*, 10, pp.S33-S38.

Watts, D.M. et al., 1987. Effect of temperature on the vector efficiency of Aedes aegypti for dengue 2 virus. *American Journal of Tropical Medicine & Hygiene,* 36(1), pp.143-152.

Waziri, N.E. et al., 2014. Evaluating a surveillance system: live-bird market surveillance for highly pathogenic avian influenza, a case study. *Pan African Medical Journal*, 18 (S1), pp.11-11.

Weather Online, 2015. [online] Available: http://www.weatheronline.co.uk/reports/climate/Indonesia.htm [accessed 11 November, 2015].

Weaver, S.C. & Reisen, W.K., 2010. Present and future arboviral threats. Antiviral Research, 85(2), pp.328-345.

Weaver, S.C., 2013. Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. *Trends in Microbiology*, 21(8), pp.360-363.

Williams, C.R. et al., 2006. Field efficacy of the BG-Sentinel compared with CDC backpack aspirators and CO2-baited EVS traps for collection of adult Aedes aegypti in Cairns, Queensland, Australia. *Journal of the American Mosquito Control Association*, 22(2), pp.296-300.

Williams, C.R. et al., 2008. The Allee effect in site choice behaviour of egg-laying dengue vector mosquitoes. *Tropical Biomedicine*, 25(2), pp.140-144.

Wilson, A.L. et al., 2014. Are topical insect repellents effective against malaria in endemic populations? A Systematic Review and Meta-analysis. *Malaria Journal*, 13(1), pp.1-9.

Wilson, A.L. et al., 2015. Evidence-based vector control? Improving the quality of vector control trials. *Trends in Parasitology*, 31(8), pp.380-390.

Wilson, A.L., Chen-Hussey, V., et al., 2014. Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. 13(1), pp.1-9.

Wilson, A.L., Dhiman, R.C., et al., 2014. Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, 8(10), pp.e3228.

Wilson, M.E. et al., 2014. Illness in travelers returned from Brazil: The geosentinel experience and implications for the 2014 FIFA World Cup and the 2016 Summer Olympics. *Clinical Infectious Diseases*, 58(10), pp.1347-1356.

Wolbers, M. et al., 2012. Considerations in the design of clinical trials to test novel entomological approaches to dengue control. *PLoS Neglected Tropical Diseases*, 6(11), pp.e1937.

WorldSat International, 2012. [online] Available at http://www.worldsat.ca.

World Health Organisation ed., 2012b. Dengue and Severe Dengue Factsheet. [online] Available at: http://www.who.int/mediacentre/factsheets/fs117/en/index.html [Accessed July 4, 2012].

World Health Organisation, 2000. Strengthening implementation of the global strategy for dengue fever/dengue haemorrhagic fever prevention and control. Report of the Informal Consultation 18-20 October 1999, Geneva: World Health Organisation. Available: http://www.who.int/csr/resources/publications/dengue/whocdsdenic20001.pdf.

World Health Organisation, 2001. FAO/WHO: Amount of poor-quality pesticides sold in developing countries alarmingly high, Press Release. Geneva: World Health Organisation.

World Health Organisation, 1971. Technical guide for a system of yellow fever surveillance. *Weekly Epidemiological Record*, (46), pp.493–504.

World Health Organisation, 1972. A system of world-wide surveillance for vectors. *Weekly Epidemiological Record*, 47, pp.73-84.

World Health Organisation, 1997. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control, Geneva: World Health Organisation.

World Health Organisation, 2009. DENGUE Guidelines for Diagnosis, Treatment, Prevention and Control. World Health Organisation Expert Meeting, pp.1-160.

World Health Organisation, 2011. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva, Switzerland: World Health Organisation; 2009 World Health Organisation.

World Health Organisation, 2012a. Global Strategy for Dengue Prevention and Control 2012 – 2020. Geneva: World Health Organisation.

World Health Organisation, 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organisation.

World Health Organisation, 2015. Integrated Vector Management, World Health Organisation. [online] Available at: http://www.who.int/neglected_diseases/vector_ecology/ivm_concept/en/ [Accessed June 24, 2015].

World Health Organisation, 2006. Pesticide evaluation scheme, 2006. 6th ed. Pesticides and Their Application. Geneva: World Health Organisation.

World Health Organisation, 2015b. WHO recommended insecticides for indoor residual spraying against malaria vectors. [online] Available: http://www.who.int/whopes/Insecticides_IRS_2_Mar_2015.pdf?ua=1. [Accessed November 11, 2015].

Xu, H.-Y. et al., 2014. Statistical modeling reveals the effect of absolute humidity on dengue in Singapore. *PLoS Neglected Tropical Diseases*, 8(5), pp.e2805.

Xu, Z. et al., 2014. Comparative analysis of the effectiveness of three immunization strategies in controlling disease outbreaks in realistic social networks. *PLoS One*, 9(5), pp.e95911.

Yap, H.H. et al., 1998. Field efficacy of a new repellent, KBR 3023, against Aedes albopictus (SKUSE) and Culex quinquefasciatus (SAY) in a tropical environment. *Journal of Vector Ecology*, 23(1), pp.62-68.

Yeo, A.S.L. et al., 2015. Dengue patients exhibit higher levels of PrM and E antibodies than their asymptomatic counterparts. *BioMed Research International*, 2015, pp.1-10.

Yi, B. et al., 2003. Relationship of dengue fever epidemic to aedes density changed by climate factors in Guangdong Province. [Chinese]. *Wei Sheng Yan Jiu*, 32(2), pp.152-154.

Yoon, I.-K. et al., 2012. Fine scale spatiotemporal clustering of dengue virus transmission in children and Aedes aegypti in rural Thai villages. PLoS Neglected Tropical Diseases 6(7), pp.e1730. doi:10.1371/journal.pntd.0001730.

Yu, K.-X. et al., 2015. Mosquitocidal and oviposition repellent activities of the extracts of seaweed bryopsis pennata on Aedes aegypti and Aedes albopictus. Molecules, 20(8), pp.14082-14102.

Zeichner, B.C. & Perich, M.J., 1999. Laboratory testing of a lethal ovitrap for Aedes aegypti. *Medical and Veterinary Entomology*, 13(3), pp.234-238.

Zhang, Y., Bi, P. & Hiller, J.E., 2008. Climate change and the transmission of vector-borne diseases: a review. *Asia-Pacific Journal of Public Health*, 20(1), pp.64-76.

Appendix 1. Data Extraction Table for Reviewed Studies.

Ref no	Author | Year | Study Pop^{n} (size + density)	Study Design | Indices | Case Definition	Objectives	Length of study and frequencies of data collection	Outcome Data / Results	Conclusions
1 8	Sanchez *et al.*, 2010. Havana, Cuba (101,484)	Case-control, block (mean = 50 houses) and neighbourhood (block plus surrounding blocks within 100m) used as units of measure BI and BI_{max} at block and neighbourhood level IgM serology.	Usefulness of larval indices (particularly BI_{max}) in identifying high-risk areas for dengue transmission for following month.	Routine surveillance data from May – July 2001 used. IgM cases reported at start of epidemic (June – August 2001). Vector data collection conducted by different vector control groups and may not have been standardized. Upon reporting of dengue cases, surveillance intensified. Calculated sensitivity, specificity PPV NPV for transmission in following month.	Of dengue cases in June, July and August, 89%, 83% and 74% lived in neighbourhoods with at least one block with BI_{max} >4 in preceding month. Sensitivity for BI_{max} in June was 81.8%. July and August was 65% and 62% respectively. PPVs for all indices were low because incidence in population was low.	BI_{max} only consistent predictor of transmission in following month. BI at neighbourhood and block level were poor predictors of dengue transmission in subsequent month, in direct contrast to Sanchez 2006 study.
2	Sanchez *et al.*, 2006. Havana, Cuba (182,485 = 5,228/km^{2})	Case-control study using residential block, neighbourhood and municipality as units BI, HI, BI_{max} (calculated at the block, neighbourhood and health area levels) IgM serology	Usefulness of larval indices (particularly BI_{max}) in identifying high-risk areas for dengue transmission	Recorded larval stage data from every house within the study area at 2-monthly intervals for 6 months. Data from 3 cycles in 2000: July-Aug (before outbreak) Sep-Oct (during) Nov-Dec (after). Dengue cases confirmed by IgM ELISA, interviewed to establish time of infection.	High correlation (r>0.94) between HI and BI; before outbreak, mean BI and HI were >1 for case neighbourhoods, <1 for controls. During outbreak mean values for case-positive blocks and neighbourhoods always higher (all p<0.05) than controls; after outbreak, indices similar.	Before outbreak, no significant ORs when predicting cases. During outbreak, all BIs (OR 3-5) significantly predicted outbreaks in blocks & presence of single positive container associated with higher risk (OR 3) for dengue transmission. Vector indices significantly correlated with positive/ negative blocks and neighbourhoods; unusual? - considerable heterogeneity between smaller areas is more typically recorded in geographical units of this size.
3 9	Chadee, 2009 Trinidad, West Indies (256, 533)	Longitudinal study of house infestations within 48hr of dengue clinical diagnosis (33 confirmed case houses), each used as an index house. One house at each cardinal point was investigated including indoor and outdoor containers. Indices compared with routine quarterly vector surveillance indices HI, BI, CI, PI, PppI Clinical WHO definition. DHF confirmed by serology, IgM.	Study conducted to determine the mosquito indices when dengue transmission occurred.	Study conducted within the months June-November 2004 (dengue season). All houses inspected quarterly (retrospective data) and within 48hrs of suspected/confirmed reported case.	Significant differences found between number of +ve houses at East and West vs. North and South. Significantly more immatures collected during case investigations than routine. 66% of index houses were +ve for larvae. HI 16% and BI 66 were much higher than routine surveillance and above 'critical thresholds'. PppI doubled during case detection.	Sample size and environment may significantly affect ability to draw concise conclusions. What can be said is that significantly higher collections occur during active entomologic surveillance vs. passive. No conclusions drawn between cases and indices.
4 10	Pham *et al.*, 2011. Dak Lak, Vietnam (1,740,000)	Ecological HI, CI, BI calculated Health stations report cases. WHO case definitions, no serology.	Describe the occurrence of dengue and its associated ecological factors (incl. indices).	Cases recorded weekly from 2004 – 2008. Entomological data collected monthly from 2004 – 2008 and then averaged across the years for each month then compared with respective dengue case data. HI and CI involved quantifying larvae <u>or</u> pupae to generate the index.	Epidemic in 2004 that accounted for 71.4% of cases. Per 5% increase, the risk of dengue incidence using univariate analysis was 1.66, 1.16, 1.78 and 1.57 for household index, household mosquito, container index and Breteau index respectively. Multivariate analysis showed increased risk at 1.87 and 1.08 for household index and household mosquito accordingly. Temperature and rainfall were 1.21 and 1.14 respectively. All figures quoted were significant at p<0.0001. From 2004 – 2008, months July through October (rainy season is May – November) accounted for 71.6% total dengue cases.	Clear associations between the various indices, seasonal climatic factors and dengue incidence. However, after multivariate analysis, only HI and household mosquito, although it is not clear what the latter is a measure of nor how it was calculated. Moreover, the RR 1.87 and 1.08 per 5% increase for each index respectively. Note that the indices included larval and pupal stages in sampling.
5	Gurtler *et al.*, 2009 Clorinda, Argentina (47,250 (2001) 49,000 (2007))	Controlled trial. Cases in neighbouring Paraguay used as external control. Reportable disease but no case definitions. Uses municipality data(?) and data from previous studies HI, BI.	City-wide control programme aimed at reducing the risk of occurrence of autochthonous cases of dengue in Clorinda using vector control strategies.	Immature inspection between Nov-Dec 2002. 14 focal intervention cycles conducted between Oct 2003 - Jun 2007 every four months. HI and BI collected at during same time period. Cases were not recorded simultaneously, and only annual data were provided by the council (suspected and confirmed) which was then retrospectively visually correlated using graphical outputs with entomological indices. Cases were confirmed with neighbouring country Paraguay over the same time period. Note: Paraguay used country statistics vs. Clorinda city data. Daily commuters crossing the border range from 2,000 to 7,000.	Neighbourhood BI decrease after first focal cycle (not significant). Conversely, HI did decrease (significant). BI distribution among taken at the neighbourhood level was highly skewed indicating focal nature/'hot spots'. 30% of variance in BI cannot be explained by covariates but due to neighbourhood-specific characteristics. Larvae/pupae were predominantly *Ae. aegypti*. Citywide HI decreased from13.7% to 3.7%, BI from 19.0 – 4.8. Monthly house and BI were highly +vely correlated over the five years (r=.966, P<0.001). Weather related variables exerted highly significant effects on larval indices, especially when time lags were allowed for. After allowing for temperature and rainfall, at p<0.001, post intervention indices declined significantly (log-transformed BI) in all cycles apart from 10 and 12 compared to pre-intervention levels. Early post-intervention surveys after cycles 1-7 showed larval indices seldom fell to 0 after treatment. Incidence of DF declined from 10.4 per 10000 in 2000 to 0 from 2001 – 2007, then increase to 4.5 in January – April 2007. Reported levels in Paraguay much higher over the same time period.	Authors conclude that larval indices could not be kept below target levels, especially during summer yet did exert a significant impact on larval population. Sustained community acceptance achieved. "Most likely" averted new outbreaks during the years 2003-2006 and limited the 2007 outbreak. Dengue cases cannot be reliably compared with Paraguay, whose reporting system may be different and whose data were for the entire country, not just one city. Entomological thresholds were consistently above 1% and 5 for HI and BI respectively.
11 6	Katyal *et al.*, 2003 Undefined sentinel sites in Delhi, India (13,782,976)	Longitudinal study HI, CI, BI calculated monthly | DF/DHF hospital case data (WHO clinical definition).	To monitor larval density of *Aedes aegypti* and dengue cases to study the trends and prevent any recurrence of an outbreak.	Monthly entomological data from 1996 - 2001. Annual incidence data until 2000 and monthly incidence during 2001.	DF/DHF cases decreased from 10252 and 423 deaths in 1996 to 180 cases and 2 deaths in 2000. HI declined from 16.1% in 1996 to 5.3% in 2000. BI and CI also showed a similar decline (no stats available for any of these figures). During 2001, increasing trend in cases (322 + 3 deaths) however falling HI (4.2%).	DF/DHF in Delhi is a local/focal phenomenon which emphasizes the need for identification of such potential disease foci. Prevention and control by health education and supported by strict legislative measures at local level can help in lowering the risk of DF/DHF. For detection of *Aedes aegypti* foci, new surveillance tools at micro level are required to be of any predictive value.
7	Chadee *et al.*, 2005 | Trinidad, West Indies, 1998	Case-control | Cases | BI	Examine the impact of routine vector control operations during the 1998 outbreak of DF/DHF.	January – December 1998 all data (clinical and lab confirmed) collected from Trinidad Public Health Lab and National Surveillance Unit of the MoH. Households visited four times per year, once every three months for inspection and treatment.	Significantly more cases in rainy season. BI around houses of 87/114 DHF cases and respective control was >5 in all but six cases. BI was >10 for 75% of DHF cases. By way of comparison, control BI averaged 5.4 +/-1.83, which was significantly lower than that for DHF cases in 84/87 cases (p<0.0001).	BI was above 5 in all counties throughout the year and increased during rainy season. Vector control failed to reduce to below this level (may be due to resistance/poor application). High density and incidence of DF was +vely correlated with peak rainfall. Suggest pre-seasonal approach at the beginning of the rainy/wet season. Provision of reliable water supply long-term goal. Protective covers may be an option. Cases were higher in those areas farther from the city centre (generally have better water supply and environmental sanitation). Adult population was high in all counties apart from 2. In these, DF remained high in spite of low BI during four inspections. Results suggest clustering or that the reports may not be representative of the county. Mosquito population feed at night. Rural and urban communities equally susceptible.

12	Rubio-Palis *et al.*, 2011 Maracay, Venezuela 1997-2005	Longitudinal Number of female Aedes per house. Use of entomological data from Urdaneta et al. (2005) – need to follow up Serology for probably case and virus isolation/RTPCR for confirmed. All data	To research the impact of the climatic variables on the cause of dengue and the abundance of *Ae. aegypti* in the metropolitan area of Maracay (AMM) for the 1997-2005 period, with the aim of generating trusted information that contributes to the design and application of strategies for the prevention and control of dengue outbreaks.	Monthly samples between Nov 2000 and Dec 2001 from the areas within and around where there were clinical cases prior to fumigating interventions.	Pearson correlation analysis showed that a positive correlation existed between rainfall and dengue cases for every year (P<0.001). Something similar is observed in the year 2001, in which exists a positive correlation between one and six weeks. Unlike the year 2005 where a positive correlation (P<0.0001) only existed in week 3 and 4.	Author: Results are not generalizable to any house but simply to those with positive cases and the houses around them in the areas considered in the study. Positive correlation between *Ae. aegypti* abundance and rainfall, noting that the largest abundance in the month of August (14.7 Aedes/house).
13	Lin & Wen, 2011 Kaohsiung/Fengshan Cities, Taiwan (Kaoh., 1.5million, Feng., 330,000)	Longitudinal. Li (lowest admin unit in Taiwan) used as unit of measure CI, BI (estimated on a monthly basis for each Li) IgM/IgG ELISA or DENV RT-PCR.	Conducted to evaluate the hypothesis that spatial heterogeneity existed for dengue-mosquito and dengue-human relationships.	On average, larval habitats in each Li were surveyed once per month.	Ordinary least squares (OLS) and geographically weighted regression (GWR) models used. 1 unit increase of BI_{max} results in 947.93 increase in average IR with OLS model. Although GWR model described the variance to a better degree no coefficients described. GWR coefficient of POPden and BI_{max} had R^2 or 0.01. R^2 was not homogeneously distributed in all Lis.	Global OLS model only explained 4% of total variance of IR. GWR model explained 59% of variance. Note that R^2 value ranged from 0.0 – 0.33, this is very broad and quite low, with the majority of areas between 0.03 – 0.18. Study provides further evidence that relationships of dengue incidence-max BI AND DENGUE INCIDENCE-POPden were spatially non-stationary. Higher human densities lead to higher incidence.
14	Chaikoolvatana *et al.*, 2007 Ubon Ratachthani, Thailand 2007 (1.6 million 103 people/km^2)	Longitudinal study CI, HI, BI ** WHO case definition for **DHF cases only** ** *** Both datasets collected by the Office of Disease Prevention and Control.*	To develop a geographical information system for surveillance of *Aedes aegypti* and dengue haemorrhagic fever in north-eastern Thailand.	Feb to Jul 2007; frequency unknown. All data collection conducted by local authorities.	No tabular data and only 4 precise figures detailing indices in specific villages. Need to correlate dengue cases and indices manually to derive meaningful data. Authors have displayed village ento data and province case data.	The results show that the number of DHF cases increased during the high disease incidence period compared to the low disease incidence period, suggesting a positive correlation between the peak rainfall period in June-July and the high density of *Ae. aegypti* mosquitoes and high incidence of DHF cases.
15	Chadee *et al.*, 2007 Trinidad 2002-2004	Longitudinal HI calculated from quarterly visits (73% of Trinidad). BI calculated from monthly visits by different team (10 houses in each county). DF and DHF cases. Blood samples taken from suspected cases for serological testing however clinical diagnoses were accepted in lieu of these tests due to workload. All suspected cases of DHF were confirmed by virus isolation or IgM.	The aim of this 3-year study was to explore the impact of climate variability on the incidences of DF and DHF and on vector densities in Trinidad.	1st Jan 2002 – 31 Dec 2004. Attempts made at inspecting all natural and artificial containers in every house and compound in Trinidad.	Warm seasons in 2002 and 2003 but not 2004 were significantly associated with relatively high BI and peaks in incidence of DF. Majority of cases 80%, 80.9% and 79.4% recorded in 2002, 2003 and 2004 respectively, occurred during rainy season p<0.001. Level of dengue-virus transmission to humans peaked as rainfall peaked in 2002 and 2003, but links were less obvious in 2004. 99% of mosquitoes were aegypti. Mean HI were 11.7, 16.3 and 13.5 for respective years. Mean BI was 29.75, 31.42 and 36.3 for 2002/3/4 respectively. BI during wet seasons significantly higher than those recorded in dry. BI during wet and dry seasons of 2004 were higher than those observed during previous years, even though incidence of DF was much lower in 2004 (p<0.001).	Most (80%) of the DF cases recorded during the present study were reported during the rainy season when the BI for Ae. aegypti ranged between 20 and 46 — that is, four to nine times higher than the BI of 5 thought to represent the threshold for dengue transmission (Macdonald, 1956). The generally high monthly BI recorded in Trinidad also indicate that the numbers of *Ae. aegypti* pupae/person also probably exceeded the threshold for dengue transmission (of about 0.25 pupa/person; Focks et al., 2000) and that the mosquito densities recorded in 2002–2004 were markedly higher than those reported in Trinidad in the 1990s (Focks and Chadee, 1997). The high mosquito densities probably contributed to the 2002 outbreak of dengue in Trinidad. The present results show no evidence of a fall in vector numbers as the incidence of DF fell between 2002 and 2004. On the contrary, the BI recorded for Ae. aegypti, which increased from 29.7 in 2002 to 36.3 in 2004, indicated that, despite the treatment of tens of thousands of containers with temephos, vector densities increased over this period (Fig. 2).

16	Correa *et al.*, 2005 Belo Horizonte, Brazil	Ecological HI (obtained from external source - service for the control of zoonoses) IgM virus isolation and PCR	To analyse the association between disease incidence and vector infestation; adopted an ecological approach using spatial areas comprising sanitary districts and basic health units.	Results of HI were aggregated by sanitary district and municipality. 17 surveys during 1996 – 2001. Results were aggregated into 4 groups, Group 1 was the value of HI below the first quartile. Group 2 the PI was equal or greater than the first quartile and below the median. Group 3 the PI was equal to or greater than the median below the 3rd quartile. Group 4 PI was equal to or greater than the 3rd quartile. The first group was considered low risk for the transmission of disease being used as the baseline. For comparison of mean of incidence in the 4 groups of PI, study used incidence of disease compared to the following month of survey.	In the first 2 years analysed, the majority of cases occurred in the district of Venda Nova. In the first semester of 1996, 50% equal to 60 areas of coverage presented PI greater than 3.9%. And in the second semester of that year, 75% equal to 80 areas of coverage had the PI below 1.5%. In the vector survey done between Feb and April of 1997 it was observed that 505 equal to 53 areas presented PI above 6.8% and only 2.8% equal to 3 areas did not have vectors identified during this survey. In the district, they observed statistically significant associations between the monthly incidence mean and the group PI p=0.02 with different statistically different between the groups 1 and 4 (p=0.02) and then between groups 2 and 4 (p=0.04). In the areas of coverage they also detect an association between the mean of incidence and the groups of PI with statistical differences between groups 1 and 3 (p= 0.01) and 1 and 4 (p=0.00).	In October 1997 no reduction in vector infestation. The PI in the district varied from 2.1% to 10.7% and 25% equal to 30 areas of coverage had the PI above 6.5%. In between June 1998 and October 2000, there was a progressive drop in the mean PI estimated for the municipality, district and health areas. In the year of 2001, the PI rose. In the present study, using data obtained from the secondary sources, positive associations were found between the intensity of vector infestation and dengue incidence in districts and areas indicating that higher PI levels were associated with a higher risk of disease incidence. It was observed that the PI values close to 1%, which is considered as low risk transmission, were associated with the occurrence of dengue in areas of coverage of health units in Belo Horizonte.
17	Fernandez *et al.*, 2005 Yurimagas, Peru. 2000-2004 (58 627)	Longitudinal study HI, CI, BI Cases confirmed by serology.	To determine the population behavior of larval *Aedes aegypti* to explain fluctuations through three entomological indexes (EI) and estimate the suspected cases of dengue in the city of Yurimaguas, Loreto, Peru between 2000 and 2004.	Apr, May, Jun, Jul, Oct, Nov (2000); Mar, Aug, Dec (2001); Apr, Aug, Dec (2002);Mar, Jul, Dec (2003); Mar, Aug, Dec (2004).	During the 2001-2004 period there were significant differences for the AI (p = 0.01), IR (F p = 0.00) and IB (p = 0.02) (Figure 2a, b, c). The 3 highest IE values was in 2000. For the three entomological indexes that showed a decreasing trend from 2000 to 2002, but a slight increase from 2003 to 2004, although this was not significant. Dengue cases registered in 60 months (2000 to 2004), decreased from 15.5 cases per month (2000) to 7.5 (2001), 3.0 (2002) and 3.91 (2003), but then there was an increase in 2004 to 10.66 cases per month. Differences exist between 2000 and 2002 (p = 0.02). No differences between the average total monthly dengue (100%) for the twelve months of the year in the study period 2000 to 2004 (p = 0.17) (Figure 3). The three entomological indexes: IA, IB and IR were highly correlated linearly and positively during the period 2000 to 2004.	Regressions were performed to estimate CDT and CDI from AI and BI, but only four regression models to estimate the CDT from monthly mean values of IA were valid, as the significance in all cases was 0.01 to 0.04 (Table 2), although the coefficients (R2) for the equations were relatively low (between 0.08 and 0.13). The linear, quadratic and cubic with and without logarithmic transformation to estimate CDI CDT and from IR and IB were insignificant (p>0.05).

<table>
<tr>
<td rowspan="2">18</td>
<td>Arboleda et al., 2012</td>
<td>Mathematical model | Presence of +ve container (+ve for immatures) in house = +ve house | BI</td>
<td rowspan="2">Evaluate the degree to which ecological niche models are able to anticipate the dynamics of breeding by Aedes aegypti, both across the region and through time, and whether breeding suitability patterns of this vector species translate into variation in human dengue case frequency through time. As part of this aim, compare the performance of niche models as predictors of human dengue case frequencies with that of</td>
<td rowspan="2">Dirección Local de Salud de Bello (DLSB) - samples of 5,709-13,137 houses randomly two to four times yearly to examine water containers and evaluate and eliminate possible mosquito breeding sites. A total of 5,709 houses was visited and sampled for mosquitoes, and 2,300 yielded records of Ae. aegypti, of which 2,075 could be georeferenced satisfactorily (i.e., to a precision of ≤20 m) over the study period.</td>
<td rowspan="2">The prediction of mosquito breeding sites can be used to anticipate concentrations of human dengue cases. Results showed a statistically significant positive correlation between area predicted as suitable by models and dengue case rates ($P < 0.05$; Table 5) in all years except 2005 and 2008. However, BI was not related significantly to dengue case rates in any year (all $P > 0.05$; Table 5). Proportional areas in each neighbourhood predicted as suitable were also not related to BI in any year (all P>0.05)</td>
<td rowspan="2">Although both niche modeling algorithms performed well in these initial tests, comparative studies of Maxent and GARP have shown that results differ at some level (Peterson et al. 2007); however, areas predicted as suitable by the two algorithms coincided closely, at least in broad outlines. In 2008, the two models matched only by 44.3%; however, for the other years, the match ranged 67.2- 76.1%. The consensus map based on predictions from all years showed that only 13.4% of Belo was consistently suitable for Ae. aegypti breeding through time. The predictions were good within initial years (i.e., from one year to the next, or to a second-third year); for predictions that were more distant in time, we obtained higher omission values, suggesting some degree of overfitting, particularly for Maxent models. This generally good predictivity opens the possibility of anticipating spatial patterns of dengue vector breeding from one year to the next by means of ecological niche modeling.</td>
</tr>
<tr>
<td>Belo, Antioquia, Colombia | 371,973</td>
<td>Dengue case information provided by local health entities with positive cases those 'that met WHO definitions as at least “suspected” cases (WHO 1997)'.</td>
</tr>
</table>

Appendix 2. Assessment of the validity of reviewed studies: Table of bias and QATQS (Quality Assessment Tool for Quantitative Studies) rating for each study.

Ref no.	Study	Study Design	Risk of Bias	Actual Bias	Comments	QATQS Rating (1>3)
1	Sanchez *et al.*, 2010.	Case-control	High	Blocks inspected by different technicians, non-standardised procedures, intensification of activities during outbreak. Misclassification of controls: underreporting could have led to occurrence of underreported/ subclinical infections in selected control blocks/neighbourhoods.	Unclear study design	3
2	Sanchez *et al.*, 2006.	Case-control	Medium	Controls: underreporting could have led to occurrence of underreported/ subclinical infections in selected control blocks/ neighbourhoods.		2
3	Chadee, 2009	Longitudinal	High	Observer bias - cases taken from various registers and GPs. No Kappa coefficient generated.	Results presented are very likely to have biased during sampling	3
4	Pham *et al.*, 2011.	Ecological	High	Risk of under/ overestimation of dengue cases		3
5	Gurtler *et al.*, 2009	Controlled Trial	High	External control was from different country with unknown reporting system	Inappropriate control group	3
6	Katyal *et al.*, 2003	Longitudinal	High	Information bias: mismatch in dengue and entomological data	No statistical analysis	3
7	Chadee *et al.*, 2005	Case-control	Low	Information bias: mismatch of data		1
8	Romero-Vivas & Falconar, 2005	Longitudinal	Medium	Information bias: use of "dengue-like" case definition likely to over-estimate actual number.	Very low number of confirmed dengue cases.	2
9	Foo *et al.*, 1985	Longitudinal	High	Selection bias - only cases from medical research establishments reported. Confounders present.	Final data on positive correlation between HI and BI was insignificant at .05 level. Unknown r^2. Rainfall and dengue incidence model was based on excessively over-estimated Aedes lifespan of 3 months.	3
10	Sulaiman *et al.*, 1996	Longitudinal	High	Pooled all Aedes species.	Study assumed homogeneity across zones.	3
11	Honorio *et al.*, 2009	Longitudinal	High	Confounding - large differences between populations, non-randomisation in selecting further participants	Households in both ento and serological surveys not matched. Small sample size for serologically-confirmed dengue infections limits statistically valid conclusions.	3

12	Rubio-Palis *et al.*, 2011	Longitudinal	High	No standard sample size for mosquito surveys - results are an estimate		3
13	Lin & Wen, 2011	Longitudinal	High	Confounding - large differences between populations may have existed; limited data available on selecting from population; did sample population correspond geogaphically with dengue cases (hospital and self reported so not necessarily from survey area)?	Although actual data used to model the associations between variables, the conclusions' strength drawn remain questionable as the variance is unexplained by the model (OLS, 4%, GWR 59%); also R^2 values ranged widely and (largely) low suggestinging substantial spatial variation and weak correlation.	3
14	Chaikoolvatana *et al.*, 2007	Longitudinal	High	Confounding - differences between populations reporting dengue and location of entomological surveys.	Data collection from primary care units, hospitals or central hospital - potential for duplication (no data tables). Article displays village vector data but province case data.	3
15	Chadee *et al.*, 2007	Longitudinal	High	Only some dengue cases recorded based on WHO definition = information bias. Surveys were population based, but dengue reports were clinically routed = potential confounding.	Due to inundation, DF cases were considered confirmed if diagnosed by clinician; DHF cases were serologically confirmed.	3
16	Correa *et al.*, 2005	Ecological	High	Selection bias - only cases from medical research establishments reported. Confounders present.	Cannot reliably correlate entomological indices with dengue cases as both areas where surveys and incidence occurred were most likely different.	3
17	Fernandez *et al.*, 2005	Longitudinal	High	Selection bias - only cases from medical research establishments reported. Confounders present - rural and urban populations.		3
18	Arboleda *et al.*, 2012	Model	High	Breteau indices generated differ temporally and spatially. Systematic error in grouping all data points into one year. Dengue cases were at neighbourhood level and therefore could not be reliably matched to indices generated throughout the year.		3

Appendix 3. Data Extraction for Reviewed Studies**.**

Ref No	Author | Year | Study Pop^n (size + density)	Study Design | Indices & Case Definition	Objectives	Length of study | Frequencies of data collection	Control Measures	Outcome Data / Results	Conclusions
1	L. Sanchez 2008 | Havana, Cuba	Intervention study | BI | IgM and clinical diagnosis	To document the process and analyze the results of implementing a strategy aimed at increasing community participation in the fight against the dengue mosquito vector.	Two years, May 2002 - May 2004 |	Campaign promoting community clean up, covering of water tanks, cleaning of vacant lots and common areas, distribution of promotional materials and housing inspections as conducted by members of the intervention community health group.	Presence of aedes in homes reduced by 79%.	Author comments: Results demostrated the effect of community led mosquito control approaches that led to a decrease in indicies by 79%. Reviewer comments: No analytic stats present, paucity in available data and outcome measures limited in number and description. Majority of paper focusses on qualitative outcomes, mainly questionnaire feedback.
2	J. Hanna | 2001 | North Queensland	Longitudinal | Ovitrap Data + BI | IgM, RtPCR or HIA	1) Descrbe epidemic and its influence on dengue prevention and control strategies in north Queensland	8 months	Clean-up campaign within 200m of case house	498 confirmed cases, median interval between symptom onset and nofication was 7 days. 7847 houses inspected, mean index was 18, 31, and 45 for Cairns, Port Douglas and Mossman respectively. 43% containers were garden items and garbage. Tyres and gully traps also common sources. 12% of ovitraps in treated areas were positive vs 27% in untreated areas (significant at p<0.05). Mean number of eggs was also significantly lower in intervention area vs untreated area.	Author comments: 8 small foci of transmission with between 2-12 cases each. Larval control and IRS resulted in lower significantly lower ovitrap catches. Immediate response to IgM mandated due to 8 day delay in awaiting confirmatory tests. Control measures commensed too late. Defined 'ignition' and 'disperal' premises, indicating that areas regularly frequented by large numbers of people can transmit the virus as the namesake suggests. Future response will target such premises if they are near to cases. Crytic breeding sites hampered response. Reviewer comments: Encouraging results from oviptrap data although these were not randomised and were present for a short time (3 days) thus not able to provide extended data capture.
3	V Vanlerberghe 2010 | Guantanamo, Cuba | 2000 inhabitants	RCT | Aedes infestation per cluster and per cycle, HI, BI, Pupae per Inhabitant	To assess the effectiveness of integrated community based environmental management (domiciliary and communal) compared with routine Aedes control in reducing pupal statistics as well as traditional Ae aegypti larval indices.	January 2005 - January 2006 | Ento data collection conducted in cycles of 11 days for one year	Multiple community based approach. Use of leaflets and flyers, community awareness groups t promote the following: clean up campaigns, container covers, education on the use of larvicide (and not to remove it) improving local water supplies by reparation and house visits to those with repeated Aedes infestation	A crude mid-term analysis in February 2006 showed a positive effect of the intervention. In view of this, and soaring entomolog- ical indices in Guantanamo municipality as a whole, the provin- cial health authorities decided to stop the trial and to generalise the intervention strategy to the whole city. Hence the preinter- vention period was defined as the three cycles covering Janu- ary 2005 and the end of intervention period as the three cycles covering January 2006. In January 2006, infestation levels in the intervention clus- ters were significantly lower than those in the control clusters (Table 2)—50% lower for the Breteau and house indices and 73% lower for pupae per inhabitant.The predominant breeding sites for both clusters remained the water storage containers at ground level (70–75%). The proportion of early immature stages (first and second instar larvae) increased significantly more in the intervention clusters (9% preintervention, 43% end of intervention) than in the con- trol clusters (6% and 12%; P=0.004). In the intervention area a non-significant (P=0.3) decrease in the percentage of repeat- edly positive blocks (5.8% v 3.5%) compared with a significant increase (P=0.005) in the control area (13.2% and 17.0%) was observed.	Author Comments: After one year *Aedes* foci were reduced to levels almost 50% lower in clusters where the community based environmental management strategy was embedded in the routine programme, compared with clusters that had the routine control programme alone. The difference in the number of pupae per inhabitant, a recommended indicator to measure the abundance of adult vector and the risk of dengue transmission,[25] reached 73%. Early immature stages (first and second instar larvae) were more common at the end of intervention, which indicates that breeding sites were eliminated more promptly with involvement of the community. Reviewer Comments: While the intervention was a success, it must be noted that this was a combined approach and not comprising a single intervention reliant on time and cooperation from various stakeholders

4	A. Baly 2009 | Santiago, Cuba | ~22,000	Controlled Intervention Study | House Index	Assess the cost-effectiveness of community-based vs. vertical control programme	5 years | 22 days - 11 days	Vertical aedes control consisted of entomologic surveillance and source reduction, larviciding (with temephos), selective adulticiding when *Aedes* foci were detected, providing health education, and enforcing mosquito control legislation. Intervention areas: a community-based environmental management approach was added to the routine vertical Aedes control program. Community working groups (CWGs) were set up to identify local health problems and needs and implement action plans related to Aedes control. These included: household level control of (peri-)domestic larval habitats, eliminating environmental risk in public areas, transforming garbage belts into vegetable gardens, repairing broken water pipes, sealing basements, and manufacturing water container covers.One area used formal and informal leaders and volunteers, and vertical Aedes control program staff. No financial incentives were offered to the members.	Effectiveness. No dengue transmission was detected during the study. In the implementation period, both areas showed a similar decrease from baseline in the number of *Aedes* foci (Table 3). During the follow-up period, the reductions from baseline further increased in the intervention areas, whereas in the control areas, they reverted to levels above baseline.	Author Comments: Belief that vertical control can be supplemented with community mobilisation to more effectively reduce transmission. Reviewer Comments: Non-randomised study - areas with high levels of infestation were chosen as intervention sites. Dengue vertical programme changed from 22 day inspection cycle to 11 day during study. Control areas actually saw an increase in ento indices in spite of vertical control methods
5	T. J. Victor 2002 | Tamil Nadu, India (~2600) |	Longitudinal | IgM/ IgG + dengue 2 viral antigen in mosquitoes | HI, CI, BI, 10 man hour biting density	Ascertain aeitology of two outbreaks in neighbouring villages (Kadumuchandiram (Kad.), Mampatti (Mam.)).	| Larval surveys and adult aspirations conducted	Application of temephos and environmental cleanup (emptying of containers), fogging twice per week for 6 weeks	2 weeks after larval intevention, CI dropped from 23 to 2 and HI dropped from 21 to 3 in Kad. Large drops in all indices measured	Author comments: Fogging was found to be effective; 2 weeks after larval intervention indices dropped and were found to be effective. Reviewers comments: No descriptive stats on important outcome measures. Large drop in all ento indices indicates success of programme yet no stats were performed on these data. No indication of when the ento surveys were taken and how long after the intervention they took place.
6	C. H. Wang 1994	Intervention | HI, Dengue incidence	Decrease number of cases by targeting mosquito	3 years	Source reduction, spraying, education	House index revealed drop to 4% in year 3 compared with 44% pre-intervention. Additionally, cases dropped from 1022 to 0 in 1990.	Author Comments: Excellent results, arguably even if imported cases were present there wouldn't be a subsequent outbreak. Reviewer Comments: After a huge campaign involving 2 million households, a reduction in house index from 44% to 4% 3 years later, data gathered in the same quarter, suggests the campaign was a success. Dengue cases also dropped from over 1100 to 0 in the 3rd year, but of course unlikely that this incidence would have been maintained throughout. Nonetheless, the results are encouraging even without stats on the relationship between HI and cases.
7	D. M. Morens 1986 | Puerto Rico, 3 municipalities: Arecibo, Bayamon & Ponce | 355,000	Longitudinal | | WHO definition with clinical diagnosis+ lab confirmation using HI and viral titre levels	1) Stop epidemic 2) Characterise epidemic 3) Learn how to prevent/control future outbreaks	Questionnaire for symptomatic cases and breeding sites | Blood from volunteers before and after spraying	Truck mounted ULV and four aerial spraying rounds | Late Sept., early Oct	Large increase in cases as vector control strategies either didn't work or were implemented too late. Rebound of adult mosquitoes (data from spray catches) revealed a sharp drop on the day and bounce back within 24-48 hours. No difference, urban vs. rural Impacted on caged mosquitoes (results elsewhere) Data revealed drop in cases before spraying	Author Comments: outbreak demonstrated that infectious disease outbreaks can still occur within countries with developed infrastructure irrespective of how unlikely this appears to be. Reviewer Comments: Figures for screening are prone to bias and confounding however provide interesting evidence for the use of screens as risk reduction for dengue infection.

8	W. J. McBride 1998 | Charters Towers, Queensland, Australia | 10,000	Cross sectional | Lab diagnosis by HIA and ELISA + recall of fever-like illness during the outbreak	To assess the current risk factors for and determinants of recent symptomatic dengue infection. Special importance was attached to evaluating the effects of behavioural and household factors.	May - September 1995 (2 years after the outbreak) | One point in time	House screening, mosquito repellents, bed nets, mosquito coils and environmental clean-up.	Presence of screening and travel to a tropical country were protective against dengue 2 infection, the former being more protective at more highly significant. Household cases or cases within 2 blocks led to increased odds of infection as did presence of water tank in property or within two blocks. Use of knockdown sprays was increased odds of infection.	Author comments: House screening was major determinant of dengue infection; observation of nearby cases increasing odds is suggestive of highly focal nature and therefore targeting as such during outbreaks. Water tanks were source of breeding and increased odds of infection. Use of knockdown sprays surprising increased odds of infection, which may have been an indicator of mosquito density. Prevention of dengue epidemic should be enforced by early case detection and prevention of virus transmission. Reviewer comments: Case definition using recall of persons who may have had fever two years ago introduces bias and in addition does not discount those who may have been infected since the outbreak (via travel/ asymptomatic infection).
9	W. Swaddiwudhipong 1992	Intervention | HI, CI, BI | Cases	To reduce Aedes infestation using communityled and vertical approaches.	3 years	Source reduction, spraying, education	Reduction over time of all indices and cases until there was an outbreak in late 1990.	Author Comments: Moderate effect on vector control due to household visits by trained health staff. Reviewer comments: No signifcance attached to data but there was an overall reduction in indices across the period. Yet, a dengue outbreak occurred on the back of the interventions, sugggesting that while vector control may have been successful, there was sufficient capacity and/or introduction of new virus type/reduction in herd immunity that propogated the latter outbreak.
10	P. H. D. Kusumawathie 2009 | Degaldoruwa, Kandy District, Sri Lanka |	Intervention |Tank positivity	Determine effectiveness of plastic net covers in domestic and peridomestic ground water storage cement tanks.	One year: August 2005 - July 2006 |	92 ground water cement tanks selected (46 for nets, 46 control) | 6 months pre intervention data collection followed by 6 months with intervention | Monthyl larval surveys	Significant reduction in mean number of tanks positive for Aedes larvae between pre and post intervention as well as between arms.	Author comments: Use of water storage nets can prevent breeding of Aedes. Reviewer comments: Data collection for baseline and intervention was conducted during different times of the year which may have impacted the results. SDs seemed particularly high in control post intervention, possibly indicating large variation between tanks suggesting that some tanks better sources than others - perhaps in more favourable areas (shaded/ larger).

11	R. E. Gurtler 2009 | Clorinda, Argentina (47,250 (2001) 49,000 (2007))	Intervention Study – no control | Cases in neighbouring Paraguay (country data vs city data) used as external control, however data not comparable | Reportable disease but no case definitions. Uses municipality data(?) and data from previous studies | HI, BI.	City-wide control programme aimed at reducing the risk of occurrence of autochthonous cases of dengue in Clorinda using vector control strategies.	Immature inspection between nov-dec 2002. 14 focal intervention cycles conducted between oct 2003- june 2007 every four months. HI and BI collected at during same time period. Cases were not recorded simultaneously, only annual data were provided by the council (suspected and confirmed) which was then retrospectively visually correlated by graph with entomological indices. Cases were confirmed with a neighbouring Paraguay over the same time period. Note: Paraguay used country statistics vs Clorinda which is a city in Argentina that borders Paraguay. Daily commuters across the border ranges between 2000 – 7000.	Larvicide, source reduction, ULV and house inspections	Neighbourhood BI decrease after first focal cycle (not significant). Conversely, HI did decrease (significant). BI distribution among taken at the neigbourhood level was highly skewed indicating focal nature/'hot spots'. 30% of variance in BI cannot be explained by covariates and is due to neighbourhood- specific characteristics. Larvae/pupae were predominantly aegypti. Citywide HI decreased from13.7% to 3.7%, BI from 19.0 – 4.8. Monthly house and BI were highly +vely correlated over the fiver years (r=.966, P<0.001). Weather relted variables exerte highly significant effects on larval indices, especially when time lags were allowed for. After allowing for temperature and rainfall, at p<0.001, post intervention indices declined significantly (log-transformed BI) in all cycles apart from 10 and 12 compared to pre-intervention levels. Early post-intervention surveys after cycles 1-7 showed larval indices seldom fell to 0 after treatment. Incidence of DF dlinced frm 10.4 per 10000 in 2000 to 0 from 2001 – 2007, then increase to 4.5 in January – April 2007. Reported levels in Paraguay much higher over the same time period.	Author comments: conclude that larval indices could not be kept below target levels, especially during summer yet did extert a significant impact on larval population. Sustained community acceptance achieved. Claims to have most likely averted new outbreaks during the years 2003-2006 and limited the 2007 outbreak. Dengue cases cannot reliably compared with Paraguay, whose reporting system may be different and whose data were for the entire country. Entomological thresholds were consistenly above 1% and 5 for HI and BI respectively.Calls transmission thresholds into question. Reviewer comments: Sources of bias due to study design (external control) and population movement between two cities. Various and greater number of serotypes circulate amongst the Paraguayan population (DENV1,2,3 vs. DENV1,3).
12	Gustavo Adolfo Ávila Montes 2004 | Comayaguela, Honduras	Intervention study | BI, HI, CI	Evaluate the impact of specialised primary school course on environmental health and dengue	Eight months: April - November 2002 |	Environmental health course intended to promote environmental clean up, safe handling of water,	There was a statistically significant difference in the Breteau index values between the two control group schools and the one intervention school where the education course was implemented more adequately than in the other intervention school.	Author comments: No statistically significant difference in BI or HI for intervention and control groups however when selecting comparing only one intervention school vs. control schools, there was a statistically significant reduction in the Breteau Index. Reviewer comments: Dengue outbreak and subsequent large municipal control effort likely confounded results.
13	J. E. M. Pessanha 2009	Ecological | Case definitions adhere to SINAN as dengue is a notifiable disease | Quick Survey Index infestation levels of *A. aegypti (LIRAa)*	To evaluate the impact of the national control plan.	2003 - 2006 | Not stated	In accordance with the National Control Plan (not specified)	Statisical association between dengue incidence post implementation of the national control plan vs. pre-intervention	Author Comments: Larval levels using Quick Survey Index demonstrated that reduction goals were not fully achieved. Reviewer Comments: Very few data available limit tangible conclusions.
14	M. E. Toledo 2011 | Mariana Grajales, Santiago de Cuba	Observational | Suspected clinical case (symptoms) then confirmed via lab (IgM) was 'dengue case' | BI		One year: April 2006 - March 2007	Neighbourhood task forces (environmental clean up, garbage belts into gardens, repairing broken water pipes, lids for water containers) vs standard control measures (source reduction, larviciding, perifocal spraying, health education and enforcement of mosquito control through fines).	In control blocks onset of cases was earlier. More cases per affected block in control areas relative risk living in control block was 4.5 (sig at p<0.05). BI was 2.0 and 5.2 respectively for control and intervention areas.	Author Comments: Community involvement can have an effect on dengue transmission as well as breteau indices. Reviewer Comments: While study design prone to many biases/ confounders, there is evidence that community-based interventions may have an impact.
15	S. Murray-Smith 1996 | Charters Towers, Queensland | 10,000	Case control | Serologically confirmed (details not provided)	Case control study undertaken to determine the effect of house screening during a dengue outbreak.	N/K	House screening | N/K	91% of cases lived in unscreened houses; 39% of controls lived in unscreened houses.	Reviewer Comments: Possibly confounded by cryptic breeding sites. Author Comments comments: Insect screens are useful early in the epidemic, as in the latter stages, transmission is more likely to occur in communal areas such as workplaces. No correlation between cases and breeding sites (not all sites mapped).

16	M. Omar 2011 | Negeri Simbilan, Malaysia |	Intervention study | Clinical case definitions and lab confirmation	This field study compares the effectiveness of a modified chemical fogging against the conventional fogging of insecticide in controlling dengue outbreak.	Seven months: 7th February 2003 - 7th September 2003	Conventional (thermal chemical fogging within 200m radius from house and subsequent fogging upon lab confirmation) vs intervention (50m thermal chemical fogging and remaining 150m ultra low volume enacted when confirmed case reported.	64.3% of outbreaks controlled within 14 days compared with 92.6% of outbreaks with the modified approach during the same time period. During independent implementation of fogging measures, the modified approach controlled 100% of outbreaks vs. only 70% with the conventional method.	Author comments: Modified chemical approach was as effective as, if not more than, the conventional approach. Reviewer comments: Results dependent on very similar conditions across all outbreaks, including accessibility, wind direction, humidity, population density, reporting, incubation period etc.
17	H. H. Pai 2006 | Kaohsiung, South Taiwan	Longitudinal study | Ovitrap index	Evaluate the impact of short term community based cleanliness campaign on behaviour change and knowledge using structured questionnaire and entomological observations.	Six months: August - December 2002 | Before, during and after weeklong community based intervention	Community-based cleanup campaign - no further details provided |	Indoor ovitrap index decreased from 57.1% to 9.5% one week after the campaign then increased to 33.9% three months later (p<0.05).	Author comments: use of short term community based cleanup campaigns can significantly impact sources of dengue vectors as well as improve knowledge. Reviewer Comments: Short term awareness raising has increased the number of positive actions carried out but this is returning to baseline levels over time. Arguably, if further observations were conducted community actions would have returned to near baseline levels.
18	A. Igarashi 1997 | Cam Bin District, Hanoi	Intervention study | IgM via ELISA | Adult density index	Evaluate the impact of Olyset nets used for interior house screening as a physical barrier and insecticide.	April - December 1994 | Fornightly collection of adults and larvae.	Olyset net used to cover doorways, windows and other routes in homes.	ADI reduced to undetectable levels in intervention area during the epidemic season in marked contrast to the control areas, which saw an increase from 0.68 - 2.0. Larval data not given. No DF/DHF cases reported in either arm. Increase in number of positive cases (33%) in control area compared to intervention (6.4%).	Author comments: Olyset Net did not show capability to effectively prevent virus transmission. Silent transmission taking place inspite of the absence of cases in either area. Reviewer Comments: Further testing of nets needed to quantify effectiveness against virus transmission - focus on materials that are more likely to interrupt transmission due to time of day used.
19	L Sanchez 2009 | Havana, Cuba | ~57,000	Longitundinal study | BI	Investigate the success/failure of increasing intersectoral collaboration in response to dengue oubreaks and management	Interventions implemented gradually in various areas over a period of six years from January 2000 - 2005	Environmental clean-up campaigns, increase communication and community mobilisation and covering water tanks including short courses for health care professionals on dengue prevention/management vs. routine control measures (entomological surveillance, source reduction, temephos application, adulticides, health education and legislation.	Dramatic reduction in BI from ~1 to ~0.05 over first two years with slight increase by the end of the intervention. However, intervention areas still remained markedly lower than baseline by the end of the intervention, similar to the levels in the control group.	Author comments: In this study, inter- sectoral coordination boosted the implementation of sanitation activities and improved community organiza- tion and participation for dengue prevention. Our results also suggest the impor- tance of an improved capacity to learn from experience, self-reflection, and to move from vertical pedagogy schemes to more participatory ones. Reviewer Comments: Non-randomised allocation of controls and intervention areas. No figures have been given, only broad statements with associated p-values.
20	S. T. Pinho 2010 | Salvador, Brazil	Mathematical model using field data | R0	Our aim is to analyse comparatively the dynamics of both dengue outbreaks in order to investigate the effect of vector control and the susceptible population pool on the reduction in the intensity and duration of the epidemics.	N/A	Adulticiding, ultra low volume spraying	Impact of adulticides clear in reducing effective reproductive number to below 1 however due to continued pool of susceptibles there may be an increase in cases at a later date. Duration of epidemics remain unchanged however intensity decreases with the application of vector control.	Author comments: The value of R0 is greater than 1 for the epidemic in 1995–1996 for any chosen value of the vector control parameter, indicating that other strategies would be necessary besides the adult vector control, such as the control of the mosquito's aquatic phase, to reduce its force of infection and therefore to control the epidemic. Reviewer comments: Control effort rates not modelled which vary in the field.

21	R. Huy 2010 | Cambodia	Report | WHO clinical case definition	Evaluate the impact of a 7 year vector control campaign against the dengue vector.	8 years using national surveillance programmes, both active and sentinel.	Temephos although not all areas received treatment at same times/frequency/duration. In addition, nationwide publicity campaigns and cleanup campaigns.	No associations before or after controlling for confounders comparing campaigns with incidence.	Author comments: No associations between disease indidence and vector control campaigns. Reviewer Comments: Under and over-reporting can contribute to paucity in information. Agree with author recommendation to better quantify dengue burden using standardised outcome measures.
22	G. M. Vazquez-Prokopec 2010 | Cairns, Queensland, Australia | 140,347	Retrospective observational study | Notificable disease, IgM positive or PCR virus positive |	In the present study we analyzed the spatio-temporal pattern of a large dengue virus-2 (DENV-2) outbreak that affected the Australian city of Cairns (north Queensland) in 2003, quantified the relationship between dengue transmission and distance to the epidemic's index case (IC), **evaluated the effects of indoor residual spraying (IRS) on the odds of dengue infection**, and generated recommendations for city-wide dengue surveillance and control.	25 week epidemic period (retrospectively analysed)	IRS around neighbouring properties upon confirmation of a case.	Odds of secondary dengue infection at unsprayed premises was 2.8, p = 0.03. IRS around >60% of neighbouring houses reduced to levels below 0. When coverage in neighbouring houses was les than 40-60% odds of infection was positive.	Author comments: IRS above 60% in areas surrounding case prevents subsequent infection however partial coverage (<40%) yields low protectivity thus timely coordination and sufficient field staff are crucial. Reviewer Comments: Detailed and timely results not often seen elsewhere. Data in a coherent and effective manner.
23	T. H. Lin 1994	Intervention | BI	Reduce vector indices and dengue over a four year period.	4 years	IRS, fogging, education, source reduction	BI reduced dramatically from baseline and low level mantained throughout follow ups.	Author Comments: Use source reduction complemented by vertical approaches (space spraying) when necessary. Reviewer comments: no statistical analysis but and no idea of coverage rate, randomisation etc., however the BI was markedly lower throughout the intervention period.
24	L. S. Lloyd 1994 | Merida, Mexico | 700,000	Intervention | BI, CI	The purpose of this article is to describe the process used to develop locally appropriate educational materials for use in a community based Ae. aegypti control program		Development and implementation of educational messages to those in the target neighbourhoods.	BI remained the same within the intervention group (126 - 129) but increased significantly within the control group (113 - 151). In addition, container index increased in control group (1.2 - 1.6) whilst decreasing within intervention arm (1.5 - 1.2) however difference between these groups was not significant.	Author comments: community education program alone may not be sufficient to generate sustainable behaviour change unles other factors are taken into consideration as part of the overall strategy. Reviewer Comments: behaviour change probably requires longer-term sustained campaign and use of contextualised materials likely increased uptake and acceptibility.
25	Y. C. Ko 1992 | Kaohsiung, Taiwan | 8,880/km2	Case control study | Serology and/or virus isolation by HAI.	Investigate the circumstances of a dengue outbreak, especially the predisposing and protective factors involved.	1987 - 1988 | Data collecte once from hospital patients and matched to controls	House and workplace screening |	Results not affected by presence of breeding sites; only 6/200 employees worked in an environment where doors were screened. Screens on houses (OR: 0.37), existence of neighbouring market and open sewers/ditches (OR: 2.85) was related.	Author Comments: Poor outdoor environmental sanitation was the main predisposing factor, while screens on doors and windows were the main protective factor against dengue infection by outdoor vector. Other possible predisposing and protective factors did not seem to differ between patients' and controls' households, despite several earlier reports to the contrary. Reviewer Comments: Dengue probably contracted at home rather than workplace. Poor sanitation and house screening were main predisposing and protective factors respectively.

26	G. A. J. S. K. Jayasooriya 2009 | Kandy District, Sri Lanka | 101,677	Intervention study | No details on case definition | BI	This study was carried out to (a) identify DF/DHF risk levels of different GN areas under the jurisdiction of the Medical Officer of Health (MOH), Kadugannawa, Kandy district; and (b) determine the impact of health education and source reduction in DF/DHF "high-risk" GN areas on the overall DF/DHF burden.	January 2004 - December 2007 | 3-4 month intervals if primary survery showed absence of larve/pupae.	Health education and environmental clean-up | one occasion only	r = 0.54 between BI and DF/DHF cases. From January - July, percentage indicent contribution to Kandy district ranged from 18.8% - 37.5% before the intervention. Subsequent teduction of percentage of cases from 22.7% to 8.8% as a percentage of total cases reported. post intervention.	Reviewer comments: No analytic stats present so impossible to elicit significance of data thus widely open to interpretation. Poor use of English and not well written. Study design lacked rigour and clear paucity in information, especially surrounding case definitions. Author comments: Application of larval control measures was of utmost importance for the prevention and control of DF/DHF. Adult vector control necessary inspite of absence of larval breeding sites. Poor correlation between BI and *albopictus*. Community based vector control necessity for elimination of potential breeding sites.
27	M. E. Toledo 2007 | Santiago, Cuba | Pop not given	RCT | HI, CI	Investigate means and methods of successful approached to community participation.	Two years: 2001 - 2002 | B-monthly inspections of all indices	Community working group formed and engaged with community on issues such as: covering of water sources; not protecting artificial containers; removing abate from drinking water.	Government drastically increaesed control measures during the first quarter of 2001, these included: chemical measures (weekly adulticiding); [rovision of plastic water containers to replace defective ones. Local leaders trained to deliver dengue-related information, education and communication and to promote environmental risk reduction. All activities were implemented in all three control health areas but not in the three intervention areas.	Author Comments: Active mobilisation of the community, starting with local identification of problems and needs and supported by CWGs where the interests both of providers and users of health services were represented, led in this study to effective A. aegypti control. Behaviours at the household level changed significantly and environmental risks for the presence of the vector decreased. This was accompanied by a significant reduction in entomological indices. Reviewer Comments: Unfortunately, data confounded by government intervention.
28	B H Kay 2002	Controlled trial | Larval population as % of baseline; BI | Reported clinically diagnosed cases	To investigate the recolonisation of subterranean habitats by *Aedes aegypti*.	2 years | 3 month intervals	Mesocyclops	Positive reduction in indices in intervention arm (not statistically significant compared with control) however statisically significant compared to baseline.	These data represent a remarkable achievement in dengue vector control. The eradication at the 400-household Phan Boi village4 has not only been maintained but extended to 1,750 households in Di Su commune with the last Ae. ae- gypti (5 larvae in a vase) detected in September 1999. We now anticipate that the entire commune will achieve eradi- cation status by September 2001. At Nghia Hiep and Xuan Phong, complete control of Ae. aegypti was also achieved; that for Lac Vien, Nghia Dong and Xuan Kien reached 99.7% or better. In April 1998, 137 of 2,551 containers con- tained Ae. aegypti larvae. By March 2000, only 6 containers were positive for Ae. aegypti, and 3 of these were concrete tanks that subsequently have been retreated with Mesocy- clops. Although the project ended in March 2000, just after winter, population levels in untreated communes remained at 14.4–367.0% of the levels recorded in April 1998. Reviewer Comments: Sought clarification on data sources/ validation but no response. Cannot use data for meta-analysis. Used only Haiphong figures for BI as post treatment controls data not avaialble from other communes.

29	Arunachalam et al 2012 | Chennai City, Tamil Nadu, India	RCT| HI, BI, CI Pupae per person	To test the efficacy of netted frames to cover key containers combined with community centred ecosystem management intervention in reducing dengue entomological indices.	June 2009 - December 2010 | Evaluations at baseline, 5 months and 10 months.	Control measures for control group were standard measures by fogging and larval control. Intervention clusters received an integrated approach including provision of water container (cement tanks), use of clean up campaigns and dissemination of information to school children.| Intervention lasted 10 months in duration. | Evaulations at baseline, 5 months and 10 months.	Substantial increase in dengue understanding in intervention group. Pupae per person index reduced to 0.004 from 1.075 (p=0.02) in intervention compared with control clusters. House index reduced to 4.2%, CI to 1.05% and BI to 4.3 from baseline values of 19.6, 8.91 and 30.8 in intervention arm.	Author Comments: Reduction in vector indices achieved through community based approach that promoted interention to prevent breeding of dengue vectors, and was targeted at multiple stakeholders within communities, led to substantial reduction in the density of dengue vectors. Reviewer comments: Analysis often focusses on baseline vs. post-intervention data which may not necessarily show a true reduction in standard indices. Also, interventions are multiple and as such it is difficult to ascribe a single intervention to the perceived success of the trial.
30	Farajollahi et al 2012 | Trenton, USA | 83,000	Controlled Trial | % control	To evaluate the area-wide efficacy of nighttime ground applied vehicle mounted ULV adulticide applications of DUET against Aedes albopicuts within an urban residential community.	Weekly collection over 2 years (2009 - 2011)	Control site remained untreated vs nighttime applications of ULV in intervention clusters. | Sampling conducted weekly for 24hrs using BG sentinel traps.	Single full label rate applications of ULV insecticide resulted in 72.7% reduction when compared with mid label rate. However, duall applications at mid label vs. single application at full label resulted in 85% reduction (p=0.003).	Author Comments: Results evidence the efficacy of nighttime ULV applications in reducing Aedes aegypti populations. Dual application at half label rate are significantly more efficient at targetting the adult vector. Reviewer Comments: Study and results seem robust and evidence a significant impact against vector population. No stats conducted between controls and intervention groups which may prove more decisive.
31	Lorono-Pino 2013 | Merida, Mexico | 800,000	RCT | Number of mosquito adults	To determine the effectiveness and acceptability of ITCs and NTCs.	1 Year | Monthly mosquito collections	Insecticide treated curtains vs. Non inseticide treated curtains	Many outcomes including # infected mozzies - abundance of females 27% lower after first follow up.	Author Comments: The presence of ITCs reduced Ae. aegypti females in the homes for a short period of time after their installation and Cx. quinquefasciatus females throughout much of the study period. In the East, the number of human infections was lower, albeit not significantly lower, and the number of DENV-infected Ae. aegypti females was significantly reduced in ITC versus NTC homes. The results were not the same in the South, in which the number of DENV infected humans or infected mosquitoes did not differ significantly between ITC and NTC homes. The reasons for this difference remain to be determined. One contributing factor may have been that DENV transmission was more intense in the East than in the South, which makes it easier to statistically demonstrate a reduction in infection prevalence in an intervention study. Reviewer Comments: Study demonstrates conflicting results therefore perhaps difficult to determine a true intervention success.
32	Lenhart 2013 | Phang Nga, Thailand	RCT | CI, HI, BI, PPI	To determine the effectiveness of ITCs	1 Year	ITCs | 3, 6 and 9 months post intervention	CI, HI, BI and PPI were 1.34 (0.86, 2.90), 1.02 (0.74, 1.43), 1.11 (0.66, 1.87), 1.36 (0.78, 2.39) respectively.	Author Comments: Results did not indicate effectiveness of ITCs. Suggest reason lies with house structure that facilitated entry of mosquitoes at multiple entry points. Reviewer Comments: Study results appear valid and reliable.

33	Vanlerberghe 2013 | Laem Chabang, Thailand	RCT | HI, BI, PPI	To assess the effectieness of ITCs	18 months | Entomological collections were at 6 and 18 months	ITCs	Coverage was 70.5% vs 33.2% in October 2007 vs October 2008. Taken first results as this is when coverage was highest and is most reflective of use of the intervention. HI, BI and PPI were 39.5% (34.0%, 45.8%) | 77.6 (64.1, 93.4) | 0.57 (0.37, 0.91) at baseline respectively and then	Author Comments: The presence of ITCs can decrease the BI and PPI in a setting in which the tools are well accepted and largely used by the households, but the scale of effect depends on the coverage and the number of curtains per house attained. The outcomes of this study also demonstrated that when the ITCs are only used in a modest proportion of houses, their deploy- ment does not affect Aedes sp. infestations. Reviewer Comments: Data seem valid within the context of a well conceieved and executed trial. Used data for meta-analysis at the point which coverage is highest to provide fair evaluation of the intervention.
34	Vu Sinh Nam 2012 | Viet Nam	Controlled Trial | Larval density index, adult density index | Dengue incidence	To test effectiveness of previous 'successful' intervention in new geographic region of Viet Nam.	4 years	Mesocyclops, clean up campaigns	Reduction in indices in intervention arm however this was not statistically significant. Achieved reduction in indices compared with baseline which was statistically significant.	Author Comments: On the basis of our previous successes in northern and central Vietnam, elimination of Ae. aegypti can be achieved by these community-based programs and will result in elimina- tion of dengue. Reviewer Comments: Lack of statistical representation thorughout. X2 stats show only independence, and not what the strength of association is. Also, cannot reliably confirm whether clean up campaigns or mesocyclops caused the reduction in indices.
35	Vu Sinh Nam et al. 2005 | Viet Nam	Controlled trial | # adults, # larvae, container positivity | dengue incidence	To detail further success using the model of prioritized control based on container productivity and community-driven bio- logic control supplemented by clean up of discarded articles.	3.5 Years	Mesocyclops, clean up campaigns	*Ae. aegypti* larval populations in the three communes were reduced by approximately 90% after one year, by 92.3−98.6% after two years of intervention, and *Ae. aegypti* populations had been eliminated from Cam Thanh and Binh Chanh with 11 larvae being detected at Ninh Xuan by June 2003. Reduction in indices in intervention arm however this was not statistically significant. Achieved reduction in indices compared with baseline which was statistically significant.	Author Comments: The community-based dengue control strategy using Mesocyclops was highly effective in controlling the dengue vector mosquito at the project sites. Specifically, Ae. aegypti were eliminated (or reduced to extremely low levels) as indicated by both larval and adult surveys. Reviewer Comments: Figures presented show dramatic reduction in most indices however statisitcal analysis inadequate for the units samples. Reductions in dengue incidence were also dramatic however no statistical outputs provided. Not possible to infer whether success was due to mesocyclops or clean up campaigns.

36	Ocampo et al. 2014	Controlled trial | CI, HI, BI, PPPI | Reported dengue incidence	Test vector control strategy	18 months | 4 entomological surveys | Incidence reported weekly	Pyriproxifen	A statistically significant reduction in the Aedes positivity of the catch basins was observed during the intervention compared to baseline. During the pre-intervention (2008–2010), Palmira reported relatively higher dengue incidence than Buga but both towns were below the 75th percentile of historic dengue cases. An epidemic from week 21 of 2009 to week 14 of 2010 was observed in Palmira. In contrast, before and during the intervention (weeks 8–35 of 2009), Buga was most of the time below the epidemic threshold except weeks 35 to 41 when a small increase in reported dengue cases was observed. When the intervention stopped (week 35 of 2009) an increase of dengue cases began to be observed in Buga reaching epidemic lev- els early in 2010. The rate ratio of dengue incidence in Buga relative to Palmira, adjusted for autocorrelation, was less in the interven- tion period, compared to the non-intervention period, by a factor of 0.19 (95% CI 0.12–0.30, p < 0.0001).The visual comparison of dengue cases against epidemic thresholds of both towns suggests that there was a delay in the onset of a dengue outbreak in Buga during the intervention period.	Author Comments: Statistical analysis estimated an approximately five-fold reduction in dengue cases during the intervention period. How- ever, this analysis cannot completely rule out causes other than our intervention for the observed patterns. The monthly treatment of catch basins with pyriproxyfen showed a significant and sustained reduction of their positivity for immature stages of Aedes. Reviewer Comments: While reduction in incidence may not be cause and effect due to study design, the evidence is suggestive that vector control did indeed impact dengue trnasmission in the intervention site.
37	Deneger et al. 2014 | Manaus, Brazil	cRCT | Adult mosquitoes, questionnaire | Serological survey	Test whether mass trapping using BGS traps would reduce *Ae. aegypti* field populations.	16 months	Biogents Sentinel Traps	Reduction in density of female aedes aegypti in intervention arm compared with control was significant at p=0.013. Subsequent measurements during dry season and second rainy season were insignificant. GAMM model for grouped data post-intervention was marginally significant at p<0.1. Serological survey indicated that transmission during the period was low and thus no significant difference between arms, however was a small but positive reduction in the odds of infection comparing houses with and without BGS traps p=0.062; OR=4.97	Author Comments: Reason for no overall effect probably due to low numbers of mosquitoes during dry season and second rainy season. Also, samples lost due to ants and power failures. No baseline serosurvey confounds results, however absence of community-wide effect t of BGS trap on reduced odds of incidence may be indiciative of localised effect (only houses with BGS traps recorded lower odds of incidence). Reviewer Comments: Well-designed study with regard to ento monitoring however lack of baseline serosurvey limits importance of serological results. BGS traps had a significant positive reduction on number of circulating mosquito adults during first rainy season, even in the presence of only 60% coverage. Further decline in significance may well be due to smaller sample size during latter seasons.

38	Tsunoda et al. 2013 | Vietnam	Controlled trial | CI, HI, PPI | Serological survey	Use of Olyset net and pyriproxifen to reduce container breeding	6 months	Olyset container net and pyriproxifen	In the trial area, containers with Olyset Net had a significantly lower percentage of Aedes immature stages positive than thosewith no Olyset Net in October. The probability of the presence of Aedes immature stages was also significantly different between containers with OlysetW Net and those without OlysetW Net, when December and February were considered together. More pupae were dead in the trial area (n=81) than in the control area (n=9) after treatment. There was no dif- ference between the trial and control areas in the effect of pyriproxyfen on the number of live Aedes pupae be- fore treatment. There were no dead pupae in containers without pyriproxyfen in the trial area, since some resi- dent forgot to keep pyriproxyfen in flower vases and ant traps. The containers treated with pyriproxyfen saw sig- nificant reduction in the number of pupae that would emerge into adults when compared to those with no treatment (χ2test; P<0.001). Seroprevalence rates of anti-dengue IgM and IgG in the healthy residents were not significantly different between the trial and control areas.	Author Comments: Our study showed that OlysetW Net and pyriproxyfen was successful in the control of Ae. aegypti immature stages, although seroprevalence rates were not signi- ficantly different between the trial and control areas. While more containers had immature mosquitoes in the trial area than in the control area before treatment, the number of positive containers and the number of pupae in the trial area were both less than in the control area after OlysetW Net and pyriproxyfen treatment, which suggests that the suppression of mosquitoes can be attributed to the treatments tested. Reviewer Comments: Effects on immature *Aedes* pronounced but no statisitcally significant data on a redction in incidence among the intervention group.
39	Castro et al. 2012 | Cuba	cRCT | BI, KAP	Use of community to bolster routine vector control interventions	27 months	The strategy included four components: setting up of organ- isation and management structures; entomological risk surveillance; capacity building at grass-root and interme- diate level; and community work for vector control.	approximately 0.1 and were comparable in intervention and control clusters. They fluctuated and showed a raising trend over time but their difference became marginally significant mid-2005 (after the intensification of A. aegypti surveillance in the intervention clusters) and substantially increased from September 2006 onwards (the launch of the CWG activities for dengue control). At the end of the observation period, A. aegypti infestation levels in the intervention clusters were substantially and significantly lower than those in the control clusters. The GEE analysis (Table 3) indicates that the difference over the intervention period was 53% (period × group interaction = 1.53, 95% CI 1.22–1.92). There were no significant differential changes in the high pre-intervention levels of knowledge of dengue symptoms and prevention measures in the intervention and control clusters (Table 2). On the other hand, good knowledge of breeding sites increased by 52.8% in the intervention clusters compared with 27.5% in the control clusters. The difference between both was significant (period × group interaction, OR 1.50). There were also no significant differential changes in the already high perception that dengue infection can be fatal.	Author Comments: The level of participation achieved, changes in preven- tive behaviours and the entomological outcome provide converging evidence of the effectiveness of the deployed community empowerment strategy. In particular, ade- quate preventive practices at the household level had increased 36% in the intervention clusters compared with no changes in the control clusters and over the 2-year intervention period the BI remained 53% lower in the former compared with the latter. Reviewer Comments: Promising approach on the augmentation of existing vector control tools coupled with strong study design.
40	Stoddard et al 2014 | Peru	Model	Secondary objective: identify reduction in transmission in years where outdoor fogging was conducted	10 years	Outdoor fogging	Cases were reduced after citywide insecticide fumigation if conducted early in the transmission season. Vector control operations did, however, appear to have a significant impact on transmission some years.	Author Comments: results indicate that vector control efforts, albeit intensive, can reduce transmission if timed and placed properly. This indicates that vector control can be an effective tool for preventing dengue. Reviewer Comments: As the authors acknowledge, statistical significance for the evdience of effective vector control in the reduction of dengue incidence is limited, even if the control tools show potential.

41	Harris et al. 2011 | Grand Cayman	Controlled Trial | Ovitrap index (flouresent vs. non-flourescent hatched larval ratio)	Test the effectiveness of genetically engineered mosquitoes (RIDL)	6 months	RIDL mosquitoes	Over the last 7 weeks of the release period, the mean ovitrap index in the untreated areas was 49% (95% CI 43–55%. In contrast, the mean ovitrap index in area A was 10% (95% CI 7–14%), which is an 80% reduction relative to the untreated areas, indicating strong population suppression in the treated area during this period.	Author Comments: The positive outcome and successful demonstration of population suppression is encouraging for genetic control strategies in general and, in particular, validates the potential of OX513A RIDL mosquitoes for population suppression. Reviewer Comments: Dramatic reduction in ovitrap index achieved although study not randomised.

Appendix 4. Assessment of the validity of reviewed studies: Table of bias and QATQS (Quality Assessment Tool for Quantitative Studies)

Ref Number	Author, Year	Study Design	Risk of Bias	Actual Bias	Methods undertaken to limit bias	Comments	QATQS Rating
1	Sanchez et al 2008	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Standardised methodology		3
2	Hanna et al 2001	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias	None reported	Descriptive study with some useful ovitrap data, if limited.	2
4	Baly et al 2009	Controlled clinical trial	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Matched on health zone	Weak study design with limited data	3
5	Victor et al 2002	Interrupted time series	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	None	No analytic stats performed on pertinent outcomes	3
6	Wang et al 1994	Before and after intervention study	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	None	Weak statistical outputs; descriptive only	3
7	Morens et al 1986	Interrupted time series	Random and Systematic Error	Selection bias, confounding	Standardized forms		3
8	McBride et al 1998	Cross sectional	Random and Systematic Error	Confounding, observer bias, recall bias	Random selection, single investigator used standardised questionnaire, logistic regression		2
9	Swaddiwudhipong et al 1992	Before and after intervention study	Random and Systematic Error	Selection bias, confounding, observer bias	None	No statistical analysis	3
11	Gurtler et al 2009	Before and after intervention study	Random and Systematic Error	Confounding, observer bias, information bias	External control, non-random	Control is based in neighbouring country with different reporting systems and demographic structure	3
12	Montes et al 2004	Before and after intervention study	Random and Systematic Error	Intervention contamination, observer bias, information bias, confounding	Quality control of intervention, matching on age and SES		3
13	Pessanha et al 2009	Ecological study	Random and Systematic Error	Confounding, observer bias, information bias	None		3
14	Toledo et al 2011	Observational	Random and Systematic Error	Selection bias, confounding, observer bias	Random selection of intervention house blocks matched on neigbourhood characteristics		2
15	Murray-Smith et al 1996	Observational	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Age and sex matched controls		2

16	Omar et al 2011	Before and after intervention study	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Random sampling		3
17	Pai et al 2006	Interrupted time series	Random and Systematic Error	Selection bias, confounding, observer bias, random error	Random sampling	Small sample sizes	2
18	Igarashi et al 1997	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias	Neighbourhood matched control	External control, non-random	3
19	Sanchez et al 2009	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias	Random sampling, structured questionnaires	Contamination/ spill over	3
20	Pinho et al 2010	N/A Model					
21	Huy et al 2010	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias	Logistic regression	Temephos distribution was measured via NDCR had intervened that year as a proxy	3
22	Vazquez-Prokopec et al 2010	Retrospective observational	Random and Systematic Error	Selection bias, confounding, observer bias	None		2
23	Lin et al 1994	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	None	No statistical analysis	3
24	Lloyd et al 1994	Interrupted time series	Random and Systematic Error	Selection bias, confounding, observer bias	Neighbourhood characteristics used to select study 'colonias'		2
25	Ko et al 1992	Case control	Random and Systematic Error	Selection bias, confounding	Age and sex matched controls, serological testing for cases and controls, blinding, records kept of loss to follow up	Sound epidemiological study	1
26	Jayasooriya et al 2009	Longitudinal	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	None	Descriptive statistics only, possibility of spill over between areas	3
28	Kay et al 2002	Controlled trial	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Standardised teaching		3
30	Farajollahi et al 2012	Controlled trial	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Matching		1
34	Vu Sinh Nam 2012	Controlled trial	Random and Systematic Error	Seletion bias, observer bias, confounding	Standardised teaching	Due to publicity it is likely that control clusters also knew of intervention	3
35	Vu Sinh Nam et al 2005	Controlled trial	Random and Systematic Error	Seletion bias, observer bias, confounding	Standardised teaching	Due to publicity it is likely that control clusters also knew of intervention	3

36	Ocampo et al 2014	Controlled Trial	Random and Systematic Error	Selection bias, confounding	Standardised data capture, randomisation amongst intervention group		2
38	Tsunoda et al 2013	Controlled Trial	Random and Systematic Error	Selection bias, confounding, observer bias, information bias	Randomisation, stratification during analysis		2
40	Stoddard et al 2014	Model	N/A Model				
41	Harris et al 2012	Controlled Trial	Random and Systematic Error	Selection bias, observer bias, confounding	None		3

Appendix 5. Summary data extraction table for reviewed studies.

Ref number	Author (year)	Location	Intervention(s)	Study design	Duration	Mosquito spp	Outcomes measured	Effective at p<0.05?
1	Sanchez *et al* 2008	Cuba	Advocacy, house inspections, community clean up, covering water tanks, environmental clean up	Longitudinal	2 years	*Aedes aegypti*	BI	No
2	Hanna *et al.* 2001	Australia	Clean up Campaign (within 200m of case house) , Larvicides, IRS	Longitudinal	8 months	*Aedes aegypti*	Dengue Incidence, Ovitraps	Yes
3	Vanlerberghe *et al.* 2010	Cuba	Community-based advocacy, awareness CWGs, clean up, container covers, education (on use of larvicide), house inspections, water pipe repair	cRCT	1 year	*Aedes aegypti*	HI, BI, PPI	Yes, all metrics
4	Baly *et al.* 2009	Cuba	Community-based environmental management, source reduction, larviciding, adulticiding, education, promote formation of CWGs, water covers	Controlled clinical trial	5 years	*Aedes aegypti*	HI	Yes
5	Victor *et al.* 2002	India	Temephos and environmental clean up, fogging every 2 weeks	Interrupted time series	N/K	*Aedes aegypti*	CI, HI, BI, Man Hour Biting Density	No
6	Wang *et al.* 1994	Singapore	Source reduction, spraying and education	Before and after intervention study	3 years	*Aedes aegypti*	HI, Dengue incidence	No
7	Morens *et al.* 1986	Puerto Rico	Truck-mounted ULV, aerial spraying x4	Interrupted time series	N/A	*Aedes aegypti*	Ovitraps, Dengue incidence	Yes ovi, No incidence
8	McBride *et al.* 1998	Australia	Knockdown Sprays | Insect Repellents | Bed Nets | Mosquito Coils | House Screening	Cross sectional	2 years	*Aedes aegypti*	Dengue incidence	Yes
9	Swaddiwudhipong *et al.* 1992	Thailand	Source reduction, spraying, education	Before and after intervention study	3 years	*Aedes aegypti*	CI, HI, BI | Dengue incidence	No
10	Kusumawathie *et al.* 2009	Sri Lanka	Water Tank Covers	RCT	1 year	*Aedes aegypti/ albopictus*	Tank Positivity	Yes
11	Gurtler *et al.* 2009	Argentina	Larvicide, source reduction, ULV and house inspections	Before and after intervention study	5 years	*Aedes aegypti/ albopictus*	HI, BI	Yes, all metrics
12	Ávila Montes *et al.* 2004	Honduras	Environmental health course intended to promote environmental clean up, safe handling of water	Before and after intervention study	8 months	*Aedes aegypti*	CI, HI, BI	Yes CI, No HI, Yes BI
13	Pessanha *et al.* 2009	Brazil	In accordance with the National Control Plan (not specified)	Ecological study	3 years	*Aedes aegypti*	Dengue incidence	No
14	Toledo *et al.* 2011	Cuba	Community-based environmental management and water covers (environmental clean up, garbage belts into gardens, water pipe repair, water container covers)	Observational	1 year	*Aedes aegypti*	BI, Dengue incidence	Yes, all metrics
15	Murray-Smith *et al.* 1996	Australia	House Screening	Observational	N/A	*Aedes aegypti*	Dengue incidence	Yes
16	Omar *et al.* 2011	Malaysia	Thermal chemical fogging and ULV	Before and after intervention study	7 months	*Aedes aegypti*	Dengue incidence	Yes
17	Pai *et al.* 2006	Taiwan	Community-based clean up campaign	Interrupted time series	6 months	*Aedes aegypti*	HI, Dengue incidence	No HI, Yes, incidence
18	Igarashi *et al.* 1997	Viet Nam	House Screening | ITMs	Longitudinal	9 months	*Aedes aegypti*	HI, ADI, Dengue incidence	Yes HI, Yes, ADI, No incidence
19	Sanchez *et al.* 2009	Cuba	Environmental clean-up campaigns, increase communication and community mobilisation and covering water tanks including education for professionals	Longitudinal	6 years	*Aedes aegypti*	BI	Yes

20	Pinho *et al.* 2010	Brazil	Ultra low volume spraying	N/A Model	N/A	*Aedes aegypti*	R0	Yes
21	Huy *et al.* 2010	Cambodia	Larviciding, advocacy and cleanup campaigns.	Longitudinal	8 years	*Aedes aegypti*	Dengue incidence	No
22	V-Prokopec *et al.* 2010	Australia	Indoor Residual Spraying	Retrospective observational	6 months	*Aedes aegypti*	Dengue incidence	Yes, all metrics
23	Lin *et al.* 1994	Taiwan	IRS, fogging, education, source reduction	Longitudinal	4 years	*Aedes aegypti*	BI	No
24	Lloyd *et al.* 1994	Mexico	Educational messages	Interrupted time series	N/K	*Aedes aegypti*	CI, BI	No
25	Ko *et al.* 1992	Taiwan	House Screening | Bed Nets | Mosquito Coils | Mosquito Traps	Case control	1 year	*Aedes aegypti*	Dengue incidence	Yes
26	Jayasooriya *et al.* 2009	Sri Lanka	Health education and environmental clean-up | one occasion only	Longitudinal	3 years	*Aedes aegypti/ albopictus*	BI, Dengue incidence	No
27	Toledo *et al.* 2007	Cuba	CWGs for covering of water sources; not protecting artificial containers; not removing abate from drinking water	cRCT	2 years	*Aedes aegypti*	CI, HI	Yes, all metrics
28	Kay *et al.* 2002	Viet Nam	*Mesocyclops*	Controlled trial	2 years	*Aedes aegypti*	CI, HI, BI	Yes, all metrics
29	Arunachalam *et al.* 2012	India	Community-based environmental management and water covers	cRCT	1.5 years	*Aedes aegypti*	CI,HI, BI, PPI	Yes
30	Farajollahi *et al.* 2012	USA	Nighttime Outdoor Fogging	Controlled trial	2 years	*Aedes aegypti/ albopictus*	% control	Yes
31	Loroño-Pino *et al.* 2013	Mexico	Insecticide-treated curtains	RCT	1 year	*Aedes aegypti*	Number of mosquito adults | Dengue incidence	No
32	Lenhart *et al.* 2013	Thailand	Insecticide-treated curtains	cRCT	1 Year	*Aedes aegypti*	CI, HI, BI, PPI	No
33	Vanlerberghe *et al.* 2013	Thailand	Insecticide-treated curtains	cRCT	18 months | Ento samples at 6, 18 months	*Aedes aegypti*	HI, BI, PPI	Yes, all metrics
34	Nam *et al.* 2012	Viet Nam	*Mesocyclops*, clean up campaigns	Controlled trial	4 years	*Aedes aegypti*	CI (absolute numbers), ADI	Yes, all metrics
35	Nam *et al.* 2005	Viet Nam	*Mesocyclops*, clean up campaigns	Controlled trial	3.5 Years	*Aedes aegypti*	Larval numbers, mosquito numbers	Yes, all metrics
36	Ocampo *et al.* 2014	Colombia	Pyriproxifen	Controlled Trial	18 months	*Aedes aegypti*	CI, HI, BI, PPPI | Dengue Incidence	Yes
37	Deneger *et al.* 2014	Brazil	BGS traps	cRCT	16 months	*Aedes aegypti*	Adult mosquitoes, questionnaire | Serological Survey	Yes
38	Tsunoda *et al.* 2013	Viet Nam	Olyset net and pyriproxifen	Controlled Trial	6 months	*Aedes aegypti*	CI, HI, PPPI	Yes up to five months
39	Castro *et al.* 2012	Cuba	Community participation to bolster existing campaigns	cRCT	27 months	*Aedes aegypti*	BI, KAP	Yes in BI
40	Stoddard *et al.* 2014	Peru	Outdoor fogging	Model	10 years	*Aedes aegypti*	Dengue incidence	No
41	Harris *et al.* 2012	Grand Cayman	RIDL mosquitoes	Controlled Trial	6 months	*Aedes aegypti*	Ovitrap index	Yes

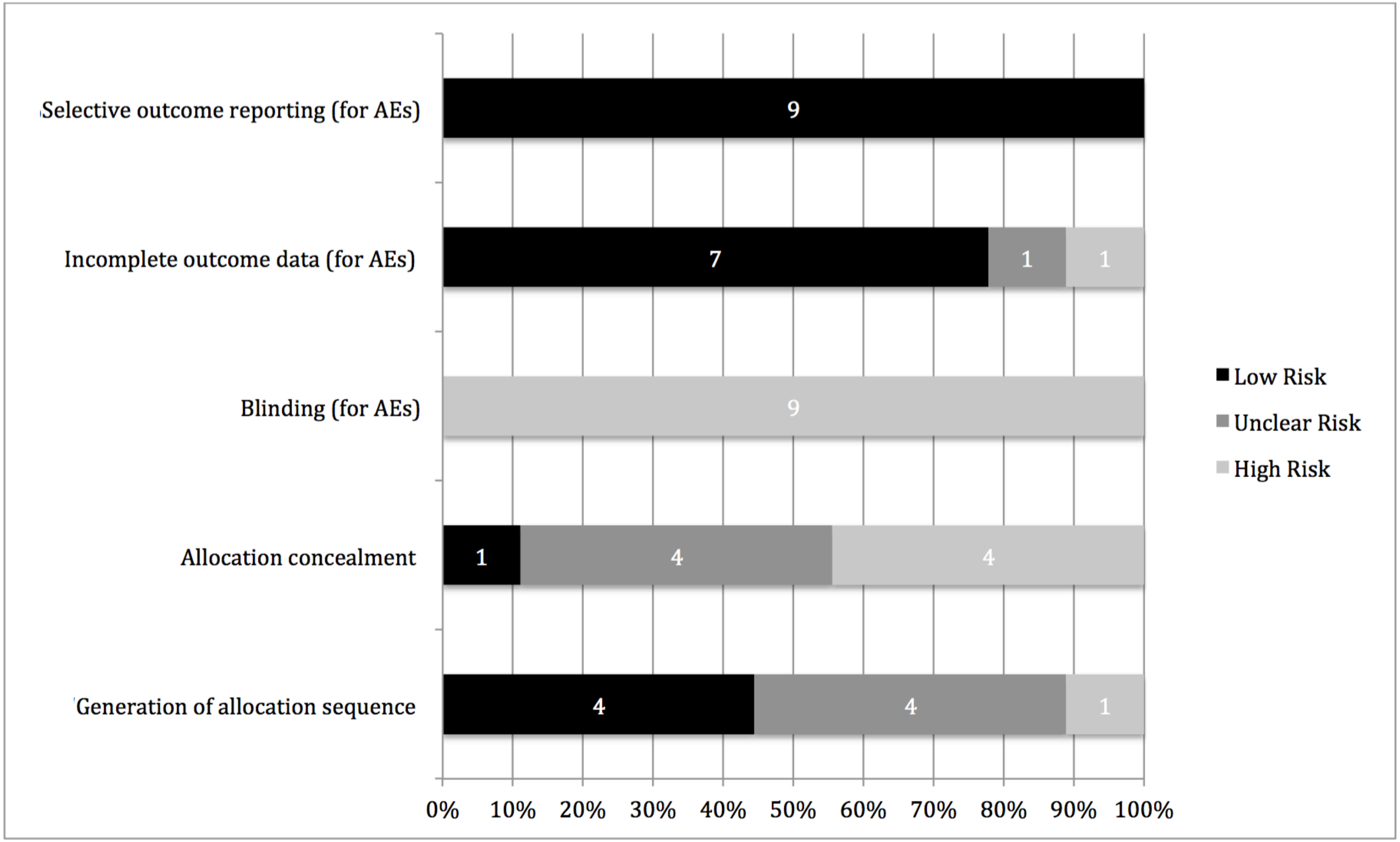
Appendix 6. Cochrane Table of Bias for Randomised Controlled Trials.

Ref Number	Trial	Generation of allocation sequence	Allocation concealment	Blinding (for AEs)	Incomplete outcome data (for AEs)	Selective outcome reporting (for AEs)
3	Vanlerberghe 2010	Low risk - drawing numbers from a bag	Unclear risk - not know whether investigator was randomising to a known group	High risk - participants knew of allocation to intervention arm	Low risk - all intervention clusters received treatment and were analysed	Low risk - could have reported container index, however reported all other indices.
10	Kusumawathie 2009	Unclear risk - sequence generation not reported, although was randomised	High risk - not known whether investigator was randomising to a known group	High risk - participants knew of allocation to intervention arm	Low risk - all tanks were monitored throughout the study	Low risk - all tank data were analysed and reported
27	Toldeo 2007	Unclear risk - randomisation occurred but method not described	Unclear risk - not known whether investigator was randomising to known group	High risk - participants knew of allocation to intervention arm	Low risk - all clusters were analysed and reported	Low risk - all outcomes were reported, although additional outcomes (HI, CI) may have been possible

29	Arunachalam et al 2012	Low risk - sequence generated using random numbers	Low risk - protocol ensured participants could not know of allocation	High risk - participants knew of allocation to intervention arm	Low risk - all clusters (households) completed the trial and data were analysed reported	Low risk - all outcome data were analysed/ reported
31	Lorono-Pino 2013	High risk - no randomisation process described	High risk - investigators knew of following household allocation	High risk - participants knew of allocation to intervention arm	High risk - number of participants with nets analysed was lower than those enrolled	Low risk - all outcomes analysed and reported
32	Lenhart 2013	Low risk - randomised lottery within strata	Unclear risk - not apparent how investigators were not aware of upcoming allocation	High risk - participants knew of allocation to intervention arm	Low risk - number of clusters maintained throughout the study, although number of households dropped throughout - this was reported	Low risk - all study outcomes analysed and reported
33	Vanlerberghe 2013	Unclear risk - reported as randomised but method not described	High risk - investigators chose controls based on proximity to intervention homes	High risk - participants knew of allocation to intervention arm	Low risk - number of households lost to follow up were low and reported	Low risk - all outcomes were analysed and reported

37	Deneger et al 2014	Low risk - households were randomised based on coin toss	High risk - investigators knew of allocation to intervention prior to coin toss	High risk - participants knew of allocation to intervention arm	Unclear risk - number of households maintained throughout not reported	Low risk - all outcomes analysed and reported
39	Castro et al 2012	Unclear - randomisation process not described	Unclear risk - allocation not described	High risk - participants knew of allocation to intervention arm	Low risk - all clusters completed the study, household exclusions reported	Low risk - all outcomes analysed and reported

Appendix 7. 100% Stacked Graph of Cochrane Table of Bias Results.



Appendix 8. Droid Survey Capacity Building Training Session

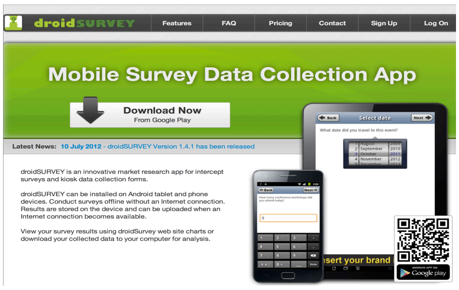
23/01/2013

An Introduction to droidSurvey

Leigh Bowman, *Liverpool School of Tropical Medicine*

LSTM

Welcome Screen

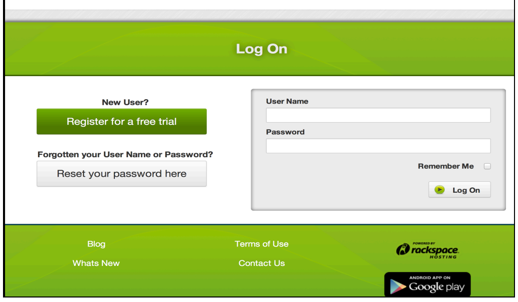


What is droidSurvey?

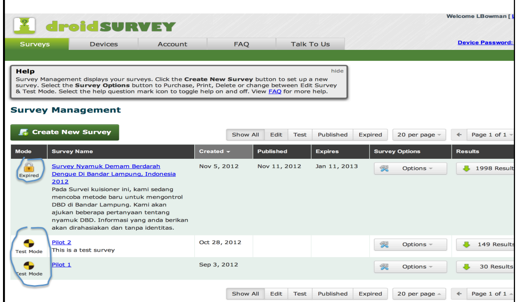
- Android-based solution
- Functions on phones and tablets
- Allow users to capture survey data
- No wireless connection needed
- All data stored on tablet until upload

LSTM

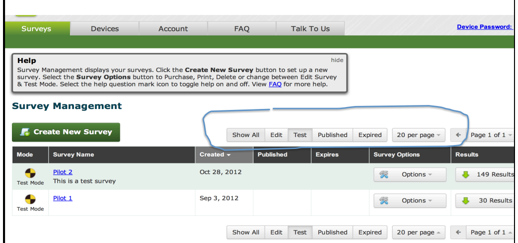
Logging On



Account Managment



Tabs



Tabs 2

- Tabs enable you to switch between modes
- Each mode allows the user to alter settings in accordance with the mode
  - Edit tab allows use to edit existing survey
  - Published tab allows user to view published surveys
  - Test tab allows user to view those surveys in 'test mode'

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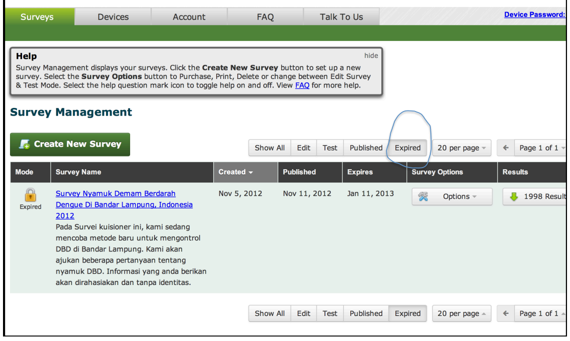
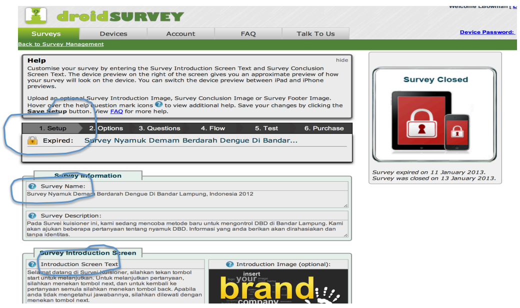
Tabs 3



Managing Surveys



Managing Surveys 2

- Click on active survey under 'Show All' tab
- Move through the flow using the tabs or button at the bottom of the screen to reach the desired category e.g. questions

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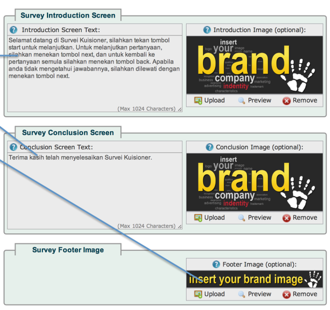
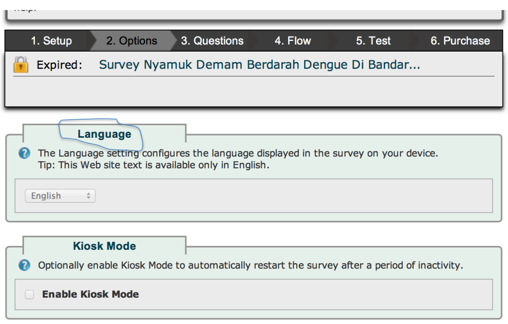
Text and Branding

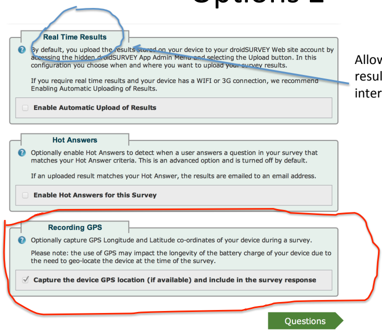
Welcome text, conclusion text and branding



Options



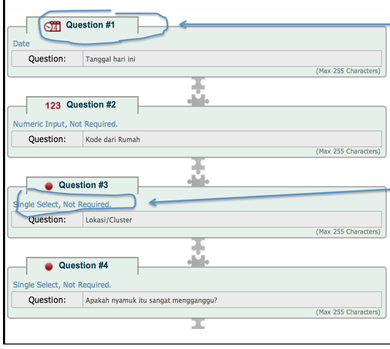
Options 2



Allows real time uploading of results NB requires active internet connection

Crucial to record the GPS position of each survey

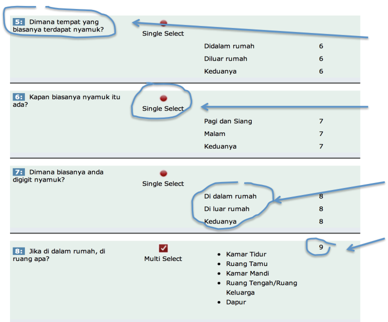
Questions



Indicates question number and flow

Indicates question type and whether mandatory

Flow



Question

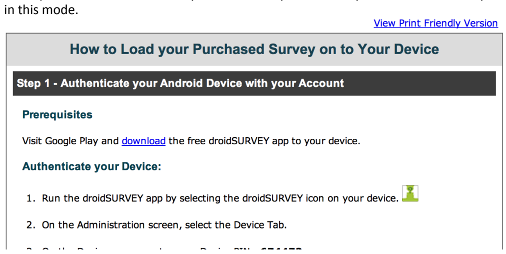
Type of answer

Available answers

Next question number

Test/Pilot Mode

Description of how to load survey to devices for pilot. NB: only 10 answers can be uploaded in this mode.



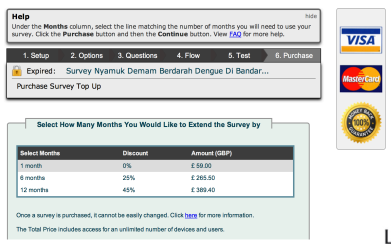
Test/Pilot Mode 2

Mock-up of how your survey will appear.

Important for picture sizing, resolution and readability

Purchase

- Various pricing options allow the user to choose the survey length required.
- Note that once a survey closes, you can still download the data up to 3 months later.
- Thereafter the dataset is no longer available so remember to download the spreadsheet!

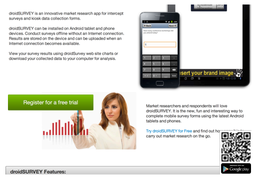


Survey Creation with droidSurvey

Leigh Bowman, Liverpool School of Tropical Medicine

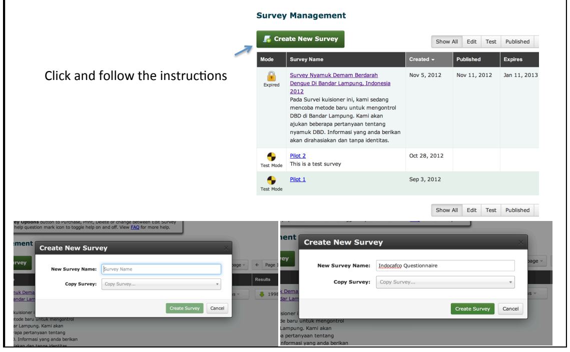
Creating an account

- As with anything, this requires a set of data to identify you and you alone.
- These data can be shared with other users to provide data sharing
  - Compromises security

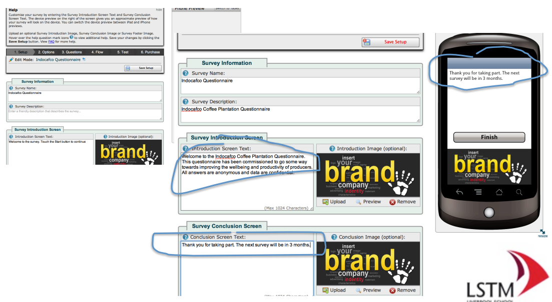


1. Create Survey

Click and follow the instructions

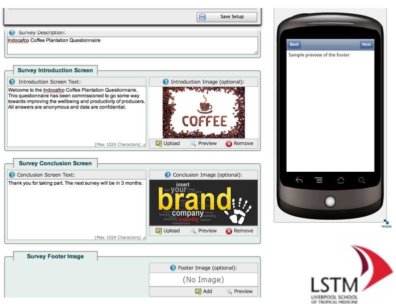


2. Opening and Closing Info

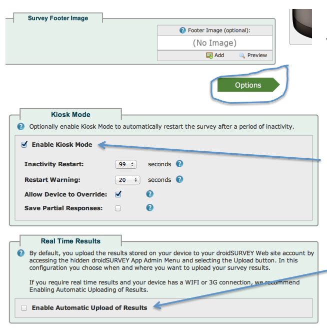


Adding Images

Straightforward instructions inform the user to search on their PC for a photo within data size limits



Options



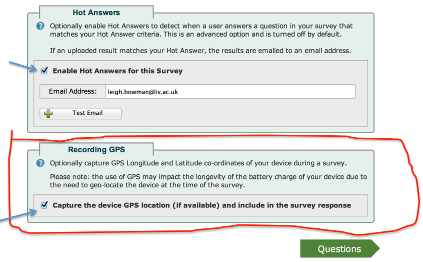
Various options are self-explanatory

I have chosen to enable so-called Kiosk Mode which helps prevent data loss

Real-time results only useful if tablet has active internet connection *at the time of taking survey*

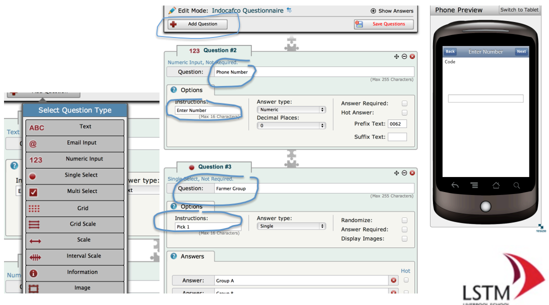
Options 2

Answers that users are particularly interested in can be emailed to notify the user immediately (once uploaded)



This is crucial for recording location!

Adding Questions



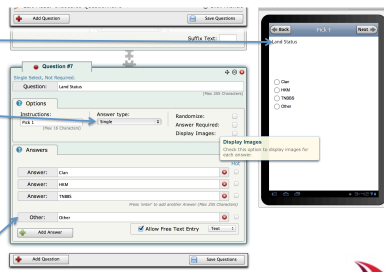
Adding Questions 2

Adding Questions 3

Updated View

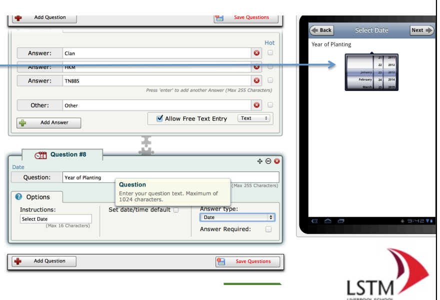
Multiple choice but *single answer only*

Note: possible to select 'other' as an option



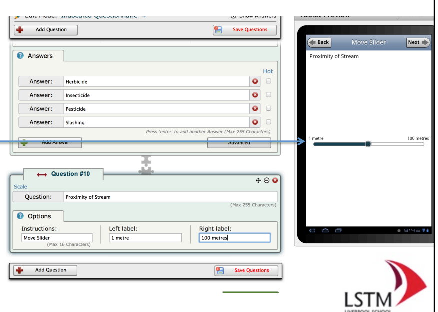
Adding Questions 4

Date entries possible (including time)



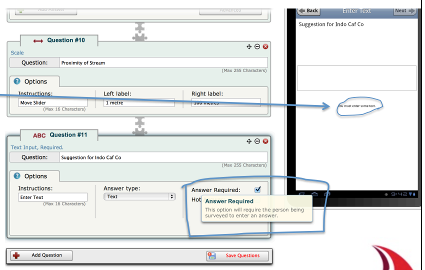
Adding Questions 5

Various question formats including sliding scales

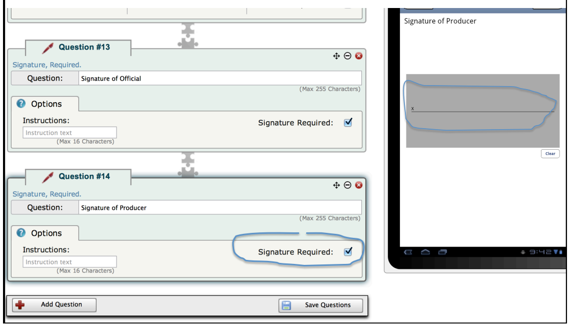


Adding Questions 6

Mandatory entry with option for user feedback



Adding Signatures



Using Flow

- Great way to visualise how the questionnaire flows
- Easy to spot mistakes
- Provides overview of the questionnaire and provides tool to change order of questions

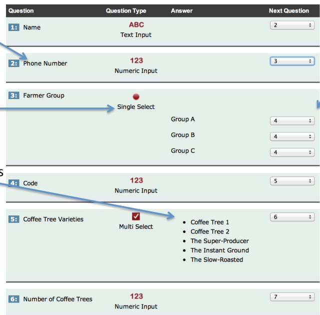
Using Flow 2

Question

Input Type

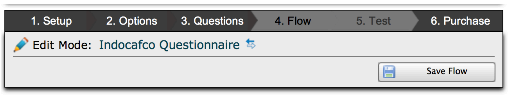
Available Answers

Next Question



Testing and Publishing

- Move the survey to 'Test Mode'

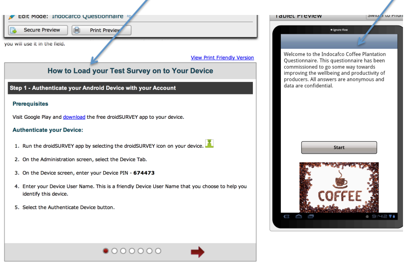


- Follow the instructions on loading to the device(s)

Testing and Publishing 2

Walk-through on how to test/publish/purchase

How your survey will appear



Go and get some data!

Thank You

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Appendix 9. Ethical Committee supporting letter for publication of Chapter 4.

LSTM
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Dr Philip J McCall
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Monday, 16 February 2015

Dear Dr McCall,

Research Protocol (12.31) Evaluation of vector control tools for dengue outbreak response

Further to your request of the 11th February 2015, the LSTM REC have concluded that it would be possible for Mr Bowman to include the ***design*** of the above study as a chapter in his PhD thesis.

However, as discussed, because of the lack of appropriate permissions none of the actual data collected can be used in this thesis or any other publication.

Yours sincerely,

Dr Angela Obasi,
Chair,
LSTM Research Ethics Committee

Appendix 10. Tables of R_2 results for calibration of outbreak probabilities by country. Top: Mean temperature vs. hospitalised cases – top 10 district R_2 values. Bottom: Total weekly rainfall vs. hospitalised cases – top 10 district R_2 values.

Brazil		Mexico		Dominican Republic		Malaysia	
District	**R_2**	**District**	**R_2**	**District**	**R_2**	**District**	**R_2**
270030	0.39	19	0.32	1102	0.71	64	0.46
520870	0.36	21	0.32	2101	0.40	34	0.43
290320	0.35	54	0.31	1302	0.35	2	0.42
150442	0.33	13	0.28	1101	0.29	56	0.41
350280	0.33	12	0.28	401	0.25	44	0.40
240800	0.32	24	0.30	1402	0.25	17	0.38
520140	0.31	38	0.28	2902	0.24	107	0.38
314800	0.31	15	0.29	3002	0.21	101	0.37
354340	0.31	11	0.30	1501	0.21	5	0.35
410830	0.29	18	0.26	1001	0.20	25	0.34

Brazil		Mexico		Dominican Republic		Malaysia	
District	**R_2**	**District**	**R_2**	**District**	**R_2**	**District**	**R_2**
251080	0.37	30	0.32	205	0.77	56	0.50
350280	0.35	19	0.32	1102	0.58	5	0.49
520870	0.32	52	0.32	1101	0.49	64	0.43
354980	0.31	10	0.30	206	0.34	25	0.40
354850	0.30	32	0.29	2601	0.31	2	0.38
320500	0.29	23	0.29	1001	0.29	107	0.37
150442	0.28	35	0.29	1501	0.28	34	0.36
500270	0.28	36	0.29	2501	0.28	17	0.36
520140	0.27	12	0.29	901	0.27	44	0.36
320530	0.27	38	0.28	1901	0.27	101	0.34

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PLOS | NEGLECTED TROPICAL DISEASES

Assessing the Relationship between Vector Indices and Dengue Transmission: A Systematic Review of the Evidence

Leigh R. Bowman[1], Silvia Runge-Ranzinger[2], P. J. McCall[1]*

1 Liverpool School of Tropical Medicine, Liverpool, United Kingdom, **2** The Special Programme for Research and Training in Tropical Diseases of the World Health Organization (WHO/TDR), Geneva, Switzerland

Abstract

Background: Despite doubts about methods used and the association between vector density and dengue transmission, routine sampling of mosquito vector populations is common in dengue-endemic countries worldwide. This study examined the evidence from published studies for the existence of any quantitative relationship between vector indices and dengue cases.

Methodology/Principal Findings: From a total of 1205 papers identified in database searches following Cochrane and PRISMA Group guidelines, 18 were included for review. Eligibility criteria included 3-month study duration and dengue case confirmation by WHO case definition and/or serology. A range of designs were seen, particularly in spatial sampling and analyses, and all but 3 were classed as weak study designs. Eleven of eighteen studies generated *Stegomyia* indices from combined larval and pupal data. Adult vector data were reported in only three studies. Of thirteen studies that investigated associations between vector indices and dengue cases, 4 reported positive correlations, 4 found no correlation and 5 reported ambiguous or inconclusive associations. Six out of 7 studies that measured Breteau Indices reported dengue transmission at levels below the currently accepted threshold of 5.

Conclusions/Significance: There was little evidence of quantifiable associations between vector indices and dengue transmission that could reliably be used for outbreak prediction. This review highlighted the need for standardized sampling protocols that adequately consider dengue spatial heterogeneity. Recommendations for more appropriately designed studies include: standardized study design to elucidate the relationship between vector abundance and dengue transmission; adult mosquito sampling should be routine; single values of Breteau or other indices are not reliable universal dengue transmission thresholds; better knowledge of vector ecology is required.

Citation: Bowman LR, Runge-Ranzinger S, McCall PJ (2014) Assessing the Relationship between Vector Indices and Dengue Transmission: A Systematic Review of the Evidence. PLoS Negl Trop Dis 8(5): e2848. doi:10.1371/journal.pntd.0002848

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Global dengue incidence has increased markedly over the past 50 years to the point where it is now the most widespread mosquito-borne arboviral disease. The World Health Organisation (WHO) has estimated that 50–100 million dengue infections occur annually, while a recent study calculated that the true figure may be closer to 400 million [1–3]. Dengue is endemic throughout the tropics, and almost half of the world's population are at risk of infection, 75% of whom live in the Asia-Pacific region [4]. Dengue has been confirmed in 128 countries worldwide [4,5] with major social and economic consequences [6–10].

Dengue is transmitted by *Aedes* mosquitoes, primarily by the highly urban-adapted vector *Aedes aegypti*, and a secondary vector *Aedes albopictus* [11]. *Ae. aegypti* thrives in the man-made urban environment, particularly in deprived communities where water storage is routine, sanitation is poor and non-biodegradable containers accumulate.

The abundance of dengue vectors species as well as dengue transmission generally show seasonal variation. Depending on the local ecology, these patterns can be in part driven by meteorological parameters such as rainfall and temperature [12,13]. Vector surveillance is recommended by WHO and is a routine practice in many dengue-endemic countries to provide a quantifiable measure of fluctuations in magnitude and geographical distribution of dengue vector populations, ultimately with the purpose of predicting outbreaks and evaluating control [14]. The standard protocol relies on the *Stegomyia* indices, which sample the immature mosquito stages (larvae and pupae) alone [15]. This approach was developed over 90 years ago [16] for yellow fever, a markedly different infection (zoonotic in origin though ultimately transmitted between humans by *Ae. aegypti*) during a very different era (*i.e.* in terms of urbanization levels and human population densities). Focks (2004) questioned the reliability and sensitivity of the *Stegomyia* indices because they correlate poorly with abundance of adult

Author Summary

Routine sampling of mosquito vector populations is common in dengue-endemic countries worldwide despite doubts about methods used or the correlation between vector density and dengue transmission. This systematic review examined the published evidence investigating associations between vector indices and dengue cases. From a total of 1205 papers identified in database searches, 18 were included for review. A range of designs were seen, particularly in spatial sampling and analyses, and all but 3 were classed as weak study designs. Thirteen studies investigated associations between vector indices and dengue cases: 4 reported positive correlations, 4 found no correlation and 5 reported ambiguous/unreliable associations. Of 7 studies that measured the Breteau Index, 6 reported dengue transmission at levels below the currently accepted threshold of 5. There was little evidence of quantifiable associations between vector indices and dengue transmission that could reliably be used to predict outbreaks. Furthermore, appropriately designed studies are required to elucidate the relationship between vector abundance and dengue transmission. Recommendations include: standardizing study designs, particularly with respect to spatial heterogeneity; vector surveillance programs should sample adult mosquitoes; global values of the Breteau Index are not reliable universal dengue transmission thresholds; and better knowledge of vector ecology is required.

mosquitoes, (*i.e.* the actual vector stage) which should be sampled directly [15]. Focks and others recommended sampling adult mosquitoes directly or indirectly via pupal/demographic surveys (calculating a pupae per person/area index, defined as the number of pupae divided by the number of residents/area surveyed) [15,17]. Indices based on actual counts of adult female *Ae. aegypti* infesting houses are likely to be the most accurate, but this is rarely done [15].

The *Stegomyia* indices remain central to the monitoring of dengue vector populations. The most commonly used indices are the House (or 'premise') index (HI - percentage of houses infested with larvae and/or pupae;) the Container index (CI - percentage of water-holding containers infested with larvae and/or pupae) and the Breteau index (BI - number of positive containers per 100 houses inspected) [14]. Variations in sampling protocols are common and can lead to significant variations in indices: *e.g.* sampling may be carried out indoors or outdoors only, or at both locations; the presence of cryptic breeding sites may lead to under-sampling or complete omission of certain sites; failure to distinguish *Aedes aegypti/albopictus* from other common mosquito species, or from each other, may lead to overestimates. Little is known about the relationship between differing proportions of the various sampled larval instars and the accuracy of these data as proxy measures of adult mosquito abundance [17]. Finally, although ovitraps (water-filled pots in which *Aedes aegypti* lay their eggs) are widely used as a simple sampling tool, Focks [15] showed very convincingly that their reliability is limited to indicating vector presence or absence.

Despite these doubts, many dengue control authorities worldwide routinely collect vector population data based on these indices, although the mathematical relationship between any of the indices and dengue transmission is far from clear. Thresholds indicating dengue outbreak risk for House and the Breteau indices (HI = 1%, BI = 5) have been used for many years [18,19], even though these values were developed for yellow fever many decades earlier. Simple thresholds may be valid in some situations [20], but a universal critical threshold applicable across many contexts, has never been determined for dengue. In pursuing the goal of identifying dengue thresholds, Scott & Morrison [21] defined the fundamental knowledge gaps as: 1) what is an acceptable level of dengue risk?; 2) what are the mosquito densities necessary to achieve that goal?; 3) what is the best way to measure entomological risk?; 4) at what geographic scale are the components of dengue transmission important? While a number of mathematical models have explored the value of thresholds or rates of change in the vector population for the prediction of dengue outbreaks [22,23], these knowledge gaps remain and continue to hinder progress [24]. For convenience, dengue outbreaks are often defined as periods when dengue incidence is equivalent to the mean plus 2 standard deviations during the same month of the previous year [25].

Effective dengue surveillance and early warning systems, using information from multiple epidemiological sources, are an important goal for numerous countries worldwide. To determine the value of vector surveillance for such systems, the findings of a systematic review examining the evidence for a relationship between mosquito indices and dengue cases are reported here.

Methods

Objectives

The aim of the study was to evaluate the potential value of vector or entomological survey data for dengue surveillance by examining the evidence from studies that investigated quantitatively the relationship between vector indices and dengue cases. The specific objectives were:

1. To identify vector surveillance methods and indices used for the routine monitoring of *Aedes aegypti* or *Aedes albopictus* populations in any geographic location.
2. To examine how entomological indices correlated with dengue incidence.
3. To examine the effectiveness or accuracy of vector surveillance in predicting dengue outbreaks and consider how this might be improved.

Search Strategy

A review protocol was established and agreed upon by all authors. Guidelines from the Cochrane Handbook for Systematic Reviews and the PRISMA Group were followed as standard methodologies [26,27]. The databases WHOLIS, PubMed, EMBASE, LILACS and Web of Science were searched using the Medical Subject Heading (MeSH) "dengue" followed by the Boolean operator "and" combined with one of each of the following 'free text' terms in succession: 'entomological surveillance', 'oviposition trap', 'house index', 'container index', 'Breteau index', 'pupal index', 'pupal survey', 'adult collection', 'sticky trap', 'aspirator collection', 'resting collection', 'landing collection', 'vector density'. The reference list of each of the included studies was also searched, and "grey literature" was sought by communication with authors for cited unpublished documents.

Results were collated in EndNote (EndNote X5, Build 7473) where abstracts were reviewed in accordance with agreed inclusion and exclusion criteria. Full text review was completed using 'Papers' (Papers 2, version 2.2.10). No limits were placed on year of publication, language or location.

Inclusion and Exclusion Criteria

The criteria for inclusion or exclusion of individual studies were set in advance (Table 1) and were used to assess each abstract and/or the full text.

Table 1. Criteria for inclusion or exclusion of studies.

Inclusion Criteria	Exclusion Criteria
Any study where entomological surveillance of *Aedes* spp. was undertaken for >3 months (or for the duration of a dengue outbreak) in conjunction with number of reported dengue cases	Studies with only one outcome of interest (entomological surveillance OR dengue cases);
Any study type with all empirical data gathered within the same time period	Opinion papers; review articles; retrospective analyses comparing data generated at different time points
Confirmed and/or probable dengue cases identified using WHO standard case definition and/or serology	Qualitative dengue reports

doi:10.1371/journal.pntd.0002848.t001

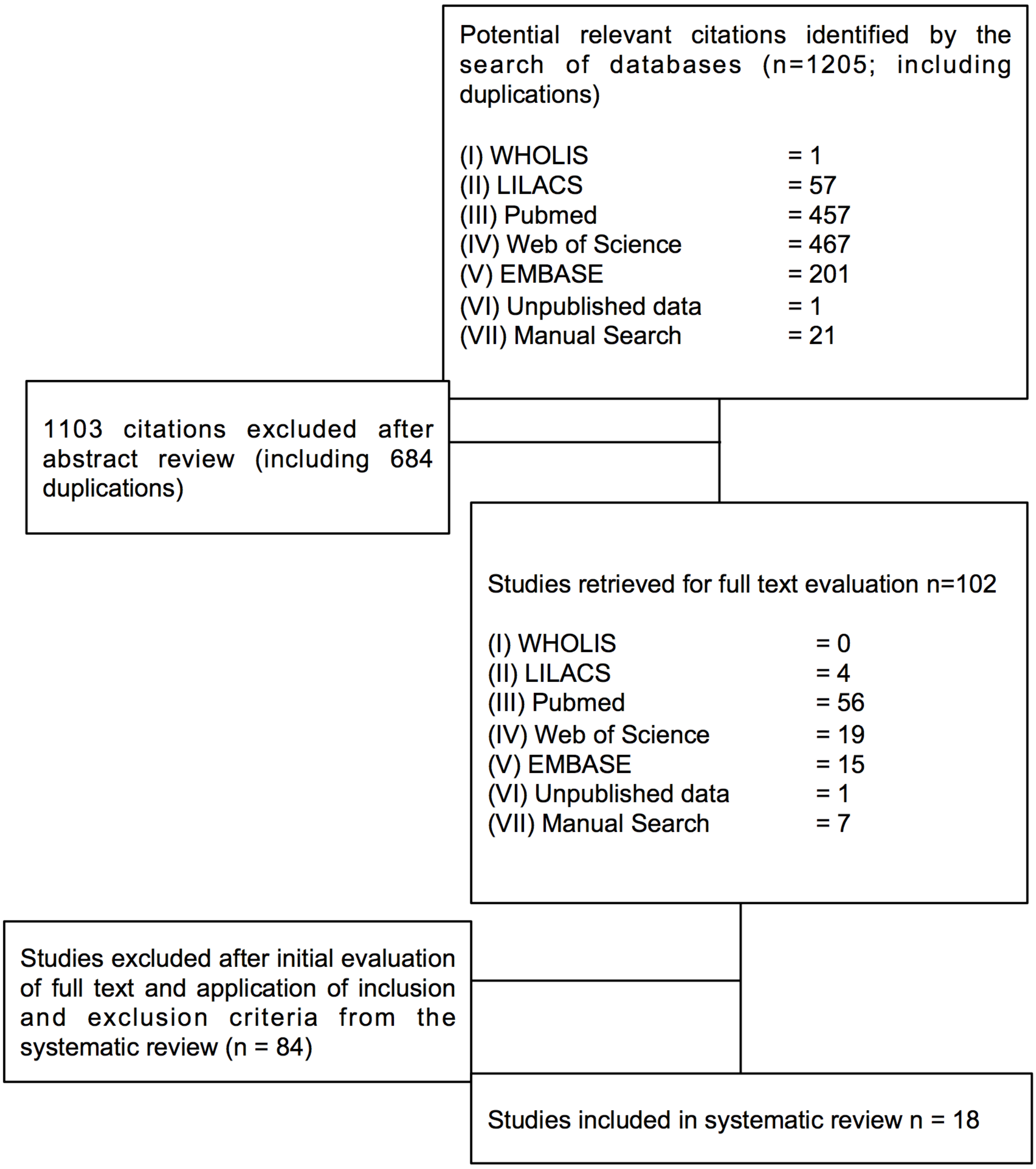

Figure 1. Search Tree. Diagram of searches performed and the number of articles returned and examined at each stage.
doi:10.1371/journal.pntd.0002848.g001

Table 2. Details of vector sampling methods used and correlation of vector indices with dengue transmission in the studies reviewed.

Ref. Number	Study	Immature Vector Indices					Adult mosquitoes sampled	Egg (ovitrap) sampled	Location		Sample spatial unit	Significant ($p\leq0.05$) increase in vector indices recorded during dengue transmission
		CI	HI	BI	BI_{max}	Pupal Index			Indoor	Indoor + Outdoor		
20	Sanchez *et al.*, 2010.			■	■		~		✓		Block; N'hood	~
29	Sanchez *et al.*, 2006.	■	■	■	■		+		✓		Block; N'hood	+
30	Chadee, 2009	■	■	■		pupae/person	+			✓	Premise	+
31	Pham *et al.*, 2011	■	■	■			+		✓		Premise	+
32	Gurtler *et al.*, 2009		■	■			~		✓		N'hood; City	~
33	Katyal *et al.*, 2003	◆	◆	◆			~					~
34	Chadee *et al.*, 2005			■			+/−			✓	Premise*	+/−
35	Romero-Vivas & Falconar, 2005	■	■	■		pupae/premise	−			✓	Premise	−
36	Foo *et al.*, 1985		◆	◆			−		✓		Premise	−
37	Sulaiman *et al.*, 1996		■⊙	■⊙			+/−		✓		City zone	+/−
38	Honorio *et al.*, 2009						−	✓			Premise	−
39	Rubio-Palis *et al.*, 2011						+		✓		Premise	+
40	Lin & Wen, 2011			■	■		+/−			✓	District; Min admin unit	+/−
41	Chaikoolvatana *et al.*, 2007	■	■	■			~			✓	Village	~
42	Chadee *et al.*, 2007		■	■			~			✓	County	~
43	Correa *et al.*, 2005						+/−				District; Trial area	+/−
44	Fernandez *et al.*, 2005	◆	◆	◆			+/−				Premise	+/−
45	Arboleda *et al.*, 2012			◆			−				0.25 km^2	−

All studies reported *Ae. aegypti* alone unless indicated otherwise. HI = House Index (% houses with larvae and/or pupae); CI = Container index (% water-holding containers with larvae or pupae); BI = Breteau index (no. positive containers per 100 houses inspected); BI_{max} is defined as the highest or 'maximum' block level BI in a neighborhood; Pupal index = pupae per person/premise defined as no. pupae divided by the number of residents/premises.
Immature vector samples are denoted as: ◆ larvae only; ■ larvae and pupae; ⊙ *Aedes aegypti* & *Aedes albopictus* combined.
Cells marked ✓ indicate the sampling activity was done.
The Sample spatial unit referred to as '**P***' is the 'premise with cardinal points index' [34]; 'N'hood' = neighborhood.
In the right-hand column, the reported association between vector indices and dengue cases is classed as: '**+**' positive association; '**−**' no association; '**+ −**' ambiguous association; '**~**' inconclusive or weak association.
Absence of any entries in cells indicates no data or information was reported.
doi:10.1371/journal.pntd.0002848.t002

Definitions

The following definition was used for the term 'vector surveillance': "Any ongoing surveillance of entomological indices, including larval indices (House Index (HI), Container Index (CI), Breteau Index (BI)), pupal indices (Pupal Productivity Index (PPI) and other variations), oviposition trap data and data from adult mosquito collections (methods include sticky, traps, CO_2, odor-baited, visual or other traps, resting catches, human landing catches), used in relation to dengue outbreak/control."

Quality Assessment

Given the strict nature of the inclusion criteria, study design was assessed at the data extraction stage using the Quality Assessment Tool for Quantitative Studies (QATQS) [28]. QATQS provides a recognized standardized method to assess study quality by assigning scores based on possible selection bias, study design, confounders, data collection methods, intervention integrity and statistical analyses. This ensured each study could be ranked qualitatively. The study design classes were intervention, case-control and longitudinal. If clarification was required, authors were contacted for any missing data or information.

Data Extraction and Assessment

The information extracted included first author, year of publication, year of study, population size, study design, indices and case definitions, study objectives, duration of study, frequency of data collection, results and conclusions (as viewed by all reviewers; Table S1). A table of bias was created to help identify the strengths and weaknesses of each study (Table S2).

Ethics Statement

No ethical review was required for this systematic literature review.

Results

A total of 1205 potentially relevant studies were identified in the database search. After reviewing abstracts, 102 were selected and retrieved for full text evaluation, of which 18 were considered to have satisfied all inclusion and exclusion criteria and explored in detail (Figure 1) [20,29–45].

Regarding the 84 studies excluded, the most common reasons for exclusion were: study duration less than 3 months (22 studies); absence of a reliable dengue case definition (21 studies); use of datasets that did not correspond temporally or spatially (19 studies). Note that although such dislocated spatial comparisons were not captured by the exclusion criteria originally defined (simply because it had not been expected), exclusion at this point was considered to be valid. Other reasons for exclusion were: measurement of only one outcome (*i.e.* vector or dengue cases only: 9 studies); opinion or review articles (8 studies); use of incomplete datasets – where only 'selected' portions of all of the data available during the study period were used (5 studies). Again, although the latter reason was not captured by the original criteria, exclusion of studies where this occurred was considered to be valid. Full details of the 18 studies reviewed are summarised in the supporting data files (Checklist S1, Table S1, Table S2).

The origin of the data used in analyses differed between studies. Some generated novel data as an integral part of the study, thus ensuring complete or independent control over the quality of the data obtained, while others obtained existing or retrospective data from external sources, including local surveillance data (*e.g.* local government records, private companies, hospitals or health centers, independent physicians and self-reported data). Twelve studies generated vector data [30–32,34–40,42–44], five generated dengue case data [29,30,35,36,38], four of which generated both vector and dengue case data [30,35,36,38].

Study Design

Fourteen studies were longitudinal, two were case-control, one was an ecological study (as defined by the unit of analysis) and one was a vector control intervention. Applying QATQS [28], fifteen studies [20,30–33,35–41,43–45] scored 3 (defined as a weak study), two studies [29,42] scored 2 (a moderate study design) and one study [34] scored 1 (a strong study design)(Annex 2). In the latter study, Chadee and colleagues [34] used controls matched on age and sex from a neighboring community, although the report did not state whether or not this process was randomized.

Vector Sampling

Details of the sampling protocols used in each study are shown in Table 2. Eleven of eighteen studies generated indices for immature stages of the vector and collected combined larval and pupal numbers to calculate either the CI, HI or BI [20,29–32,34,35,37,40,42,43]. One of these [37] combined *Ae. aegypti* and *Ae. albopictus* data. Four studies sampled only larvae [33,36,44,45].

Thirteen studies reported the location of the immature stage mosquito samples: six studies sampled both indoor and outdoor containers [30,34,35,40–42], while seven searched indoor containers only [20,29,31,32,36,37,39]. Thus, where reported, all studies included indoor sampling.

Pupal indices were reported in two studies [20,35]. Adult mosquitoes were sampled in three studies [38,39,43].

Relationship between Entomological Indices and Dengue Cases

Thirteen studies examined the association between entomological indices and dengue, using a range of different statistical approaches. Seven studies calculated regression coefficients [36,37,39,40,43–45], two calculated rate ratios [31,38], one calculated odds ratios [29] and two calculated the G-test for significance [32,36]. One study used only specificity, sensitivity and positive and negative predictive values [20].

The spatial unit of analysis, an important consideration in dengue epidemiology (see Discussion) varied considerably across studies, with units ranging from individual houses, housing blocks and clusters to neighborhoods and even large municipalities (Table 2).

Four studies reported statistically significant positive relationships between entomological indices and dengue incidence [29–31,39]. Of these, only one sampled adult mosquitoes (33% of those studies that sampled adults) [39] while the remainder sampled immature stage mosquitoes (20% of all those that sampled immatures) [29,30,31](Table 2). These are discussed in detail here.

Evidence for Positive Correlation between Vector Indices and Dengue Cases

Sanchez (2006) [29] conducted a case control study using two geographical units for analysis, blocks (units of approximately 50 houses) and neighborhoods (each containing approximately 9 blocks). Any block or neighborhood with at least 1 confirmed case was considered positive, while a control was defined as a block or neighborhood without confirmed cases. HI and BI mean values were "consistently, substantially and significantly higher" in blocks with dengue cases compared with control units. An odds ratio (OR) of 3.49 ($p<0.05$) for dengue transmission was associated with the presence of a single positive container in a block; fifteen of the seventeen dengue cases recorded lived in a neighborhood where at least 1 block had a BI>4.

In Trinidad, Chadee (2009) [30] compared retrospective routine entomological household data with concurrent entomological data taken from confirmed dengue households, using a cardinal points approach (*i.e.* the 'index' house plus the four adjacent houses at its cardinal points). Chadee found that significantly more ($P<0.001$) immatures were collected during dengue case investigations than during the routine inspection and treatment cycles. The report also stated that pupae per person indices were higher and significantly more adults emerged (as a function of total pupae count collected from household containers) at locations where dengue was confirmed at the index house, compared with routine investigations.

Pham *et al.* [31], examined monthly dengue case data, vector larval indices and meteorological data from central Vietnam, between 2004 and 2008. They found significant associations between all entomological indices and dengue cases by univariate analysis but only the HI and "household mosquito index" (not defined in the paper), temperature and rainfall were significant after multivariate analysis.

In Venezuela, Rubio-Palis *et al.* [39] used a simple regression analysis to investigate correlations between vector indices, climatic variables and dengue incidence for the period 1997–2005. Analyses indicated a significant relationship ($R^2 = 0.9369$) between the numbers of dengue cases, *Ae. aegypti* abundance (both immatures and adults) and rainfall. Acknowledging the retrospective nature of the study, the authors expressed caution in the predictive value of the findings. Moreover, another limitation was that entomological data were derived only from actual homes and neighbouring houses of confirmed dengue cases but no data were collected from 'control' houses.

Value of Vector Indices for Advance Warning of Dengue Outbreaks

Within these four studies was some additional evidence that observed changes in vector indices might be useful for the prediction of impending dengue transmission or outbreaks. In Cuba, Sanchez (2006) [29] reported that blocks with BI_{max} (defined as the highest or 'maximum' block level BI in a neighborhood) values greater than 4 were significantly more likely to record positive cases in the following month, and had a 3–5 times greater dengue risk in comparison with control blocks. The report concluded that $BI_{max}>4$ and neighborhood BI>1 during the preceding 2 months provided "good predictive discrimination". In northern Venezuela Rubio-Palis *et al.* [39] found the most significant correlation between rainfall levels and the appearance of dengue cases two months later, indicating that the magnitude of outbreaks might be predictable to some extent following periods of rainfall. Pham *et al.* [31] confirmed an association between dengue transmission and periods of higher rainfall and mosquito abundance in the central highlands of Vietnam, but did not indicate whether this could be used in advance of transmission as a predictive tool.

Unreliable or Absence of Correlation between Vector Indices and Dengue Cases

A further five studies [34,37,40,43,44] reported ambiguous evidence of associations, both positive and negative, between entomological data and dengue cases. In Belo Horizonte, Correa *et al.* [43] found a 5–7 fold increase in mean monthly dengue incidence where the 'infestation rate' (defined as house index) was "between 1.33% and 2.76% and equal to or higher than 2.77% when compared to areas showing 0.45% or less", although it was unclear whether or not this was statistically significant. They reported a moderate but significant correlation between adult *Aedes spp.* infestation rates and numbers of dengue cases ($R = 0.67$) even

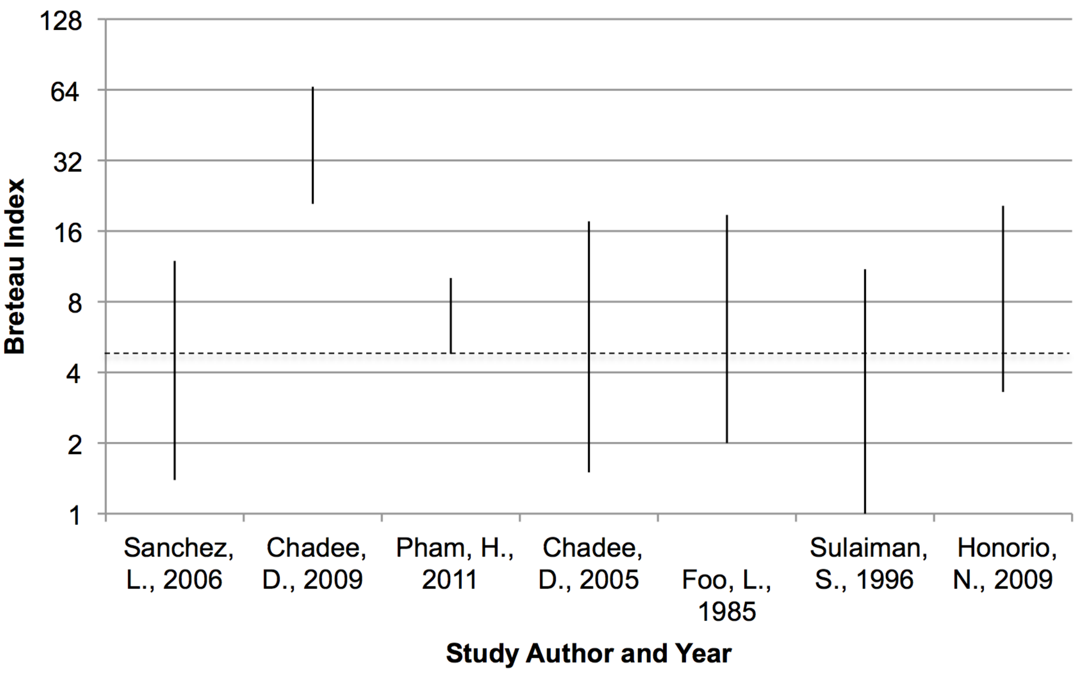

Figure 2. Range of Breteau indexes reported during dengue transmission. Dotted line indicates a BI value of 5, which has been considered a transmission threshold for dengue [21,45,52]. Note: Includes all data where available, whether statistically significant or insignificant.
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though HI and dengue cases were only weakly correlated ($R = 0.25$ at the municipal level; $R = 0.21$ and $R = 0.14$ at the district and village level). Sulaiman *et al.* [37] reported a significant correlation between BI and HI and dengue cases in certain areas of Kuala Lumpur, but not in others. In Trinidad, Chadee *et al.* [34] found that 75% of DHF cases were located in areas where BI was greater than 10, although BI and dengue infections were rarely correlated. An additional two studies reported either very low correlations between vector indices and dengue [44], or utilized highly variable inter-annual data precluding such analyses [40].

Four studies, from Malaysia [36], Brazil [35] and Colombia [35,45] found no statistically significant relationships between entomological indices and dengue cases. Foo *et al.* [36] observed a positive but non-significant association between dengue cases and HI and BI, which they suggested may have been influenced by the small sample size, the presence of *Ae. albopictus* and socio-demographic factors. Honorio *et al.* [38] found no significant associations between recent dengue cases and *Ae. aegypti* densities and proposed that infections received outside the home were responsible. In Colombia, Romero-Vivas and Falconar [35] reported distinct positive temporal correlations between the larval density index and pupal density index ($p<0.005$) and a negative association between the larval density index and egg density index ($p<0.01$); however, they found no correlation between any of the larval, pupal or adult indices with either rainfall or dengue-like cases. The spatial model of Arboleda *et al.* found no indication that the BI was in any way correlated with the dengue cases or those areas predicted as 'suitable' [45].

In the remaining studies [20,32,33,41,42] a variety of mixed, inconclusive or weak associations were reported. Gurtler *et al.* conducted analyses on the effect of a given intervention on mosquito indices but not on dengue cases [32]. Although Katyal *et al.* [33] did not present any statistical analysis, they reported the observation that over a five year period, a fall in cases was visually correlated with a fall in indices. However, they conceded that "an increasing trend of cases was observed [in 2001] in spite of a further declining HI trend", and concluded that HI had no predictive value at the 'macro' level. Despite the absence of statistical analysis, Chaikoolvatana *et al.* [41] reported a suggestive link between dengue haemorrhagic fever (DHF) during peak annual rainfall months and high abundance of mosquitoes. Chadee *et al.* observed ambiguous associations, with BI partially correlating with dengue fever cases for two out of three years [42]. As in their earlier study at the same Cuban location [29], Sanchez et al [20] reported that while $BI_{max} \geq 4$ was a useful predictor for outbreaks at the block level, sensitivity during outbreaks ranged between 62% and 81.8% and specificity between 71.9% and 78.1%.

Use of Vector Indices as Transmission Thresholds

The Breteau Index (BI) was used as an outcome measure in seven studies [29–31,34,36–38] and BI_{max} threshold was considered in three (Table 2) [20,29,40]. Here, BI values ranged from 1 to 66 during periods when dengue transmission was recorded (Figure 2). In other studies, both recent [46] and historic [47], dengue transmission was recorded when BI values were lower than the widely accepted transmission threshold of 5. Notably, in a study in Trinidad, 'high' transmission (25–40 cases for 75% of sample 'cycles') took place in areas with relatively 'low' abundance ($\sim BI<5$) while, conversely, a consistently higher BI of 5.4 in neighbouring areas did not result in dengue cases [34]. In Rio de Janeiro, the BI did not correlate with dengue incidence and transmission occurred in association with a wide range of BI levels (range 3.30–20.51) [38].

Discussion

With worldwide dengue transmission levels at an all time high, predicting dengue outbreaks in advance of their occurrence or identifying specific locations where outbreak risks are highest is of critical importance. This review considered the evidence that changes in vector populations can be correlated with dengue virus transmission and whether or not monitoring fluctuations in vector indices might be employed to provide reliable advance warning of impending dengue outbreaks.

Eighteen studies that had the potential to provide evidence of any association between vector indices and dengue incidence were identified and examined. Notably, only 4 studies utilized new data on both vector indices and dengue cases collected *de novo* as an integral part of the study. More common was a reliance on local government-level records for the dengue case data, a practice that potentially introduces error or bias for number of reasons. First, hospital reports are prone to selection bias, as asymptomatic/inapparent infections may not be recorded and the actual number of cases may have been significantly underreported. Second, there can be a considerable delay between the times of onset of infection and reporting which, if the infection date is not calculated, would result in a temporal mismatch of vector and case data. Third, differences between the geographic location of the vector and dengue case data, or between the spatial units from which each was originally calculated, would result in a geographic mismatch or mask potential relationships, respectively.

The latter point is of particular significance not only from the point of view of these studies, but also when considering the design of future investigations. A growing body of evidence indicates that the distribution of dengue cases typically is highly clustered in both time and space. In various studies, post-dating those reviewed, the size of such clusters ranged from 800 m [48] to less than 100 m [49]. The effective area of such key 'pockets' or 'hotspots' is likely to be determined by dispersal of the vector [49,50] which itself can vary over time [51], and is influenced by house density [52] and by human movement within and beyond the infection cluster [53]. Consequently, in studies attempting to correlate vector indices with dengue transmission, and where the geographical unit is too large, high vector densities in key dengue hotspots might be diluted by inclusion of neighboring areas with low densities, thus masking any true relationships [see 38].

Indeed, human movement potentially confounds dengue vector data that derive from residential areas alone as increasingly, evidence indicates that only a proportion of dengue infections are transmitted in the individual's own home, with many infections (possibly the majority) resulting from bites by virus-infected mosquitoes at other houses, schools, workplaces or numerous locations remote from the home [53,54]. Clearly, this presents a serious challenge when considering the use of vector data for surveillance and highlights a need for inclusion of data from public locations [55] in addition to residential areas, in any surveillance program.

Returning to the studies examined in this review, the fact that there was no clear indication of any consistent association between vector indices and dengue cases is not unexpected, given the diverse and mostly weak study designs. One study found there was no apparent increase in vector indices coinciding with what was the largest increase in dengue fever cases of all areas studied [40], while in another, dengue transmission remained low despite exceptionally high vector indices [44]. In studies where correlations were calculated for HI, BI and dengue cases, regression coefficients ranged from weak/moderate non-significant ($R = 0.43$ and $R = 0.35$ respectively; $p > 0.05$) [38], to moderate significant associations ($R = 0.61$ and $R = 0.60$ respectively, but only in the urban centre; $p < 0.05$) [37].

Only two studies calculated pupal indices, even though fifteen of the eighteen studies reviewed were published more than three years after WHO acknowledged that the traditional *Stegomyia* indices were inadequate for the measurement of dengue vector abundance [56]. In the two studies included in this review that calculated pupal indices, only one reported increases in the pupal index, but its relationship with dengue cases was not statistically significant, possibly due to the low numbers of pupae recorded [30,35]. A major problem with pupal surveys is the difficulty in locating breeding sites and the potential existence of important or key but cryptic breeding sites (*e.g.* overhead tanks on houses or underground water reserves such as sewers or wells) that may harbor significant proportions of the vector population [57,58].

Clearly, calculation of adult female *Aedes aegypti* indices is the most direct measure of exposure to dengue transmission [15]. Of the four studies reviewed that reported some correlation between vector indices and dengue cases, two [31,39] recorded adult vector data. The adult population of *Aedes aegypti* is rarely sampled, partly due to the erroneous but commonly held belief that carrying out such sampling is time-consuming, difficult or expensive [59].

Sampling adult female *Aedes aegypti* is a relatively simple task, though it can be limited by the fact that mosquito numbers often remain low during outbreaks [60]. Nonetheless, it is possible to aim to sample adult mosquitoes as a routine procedure with minimal additional training and resources. A number of novel sampling devices [61–63] offer the potential to monitor vectors during outbreaks [64] and at the spatial scale required to accurately sample populations of *Ae. aegypti* [65]. Simple affordable low-tech tools that enable localized sampling of adult *Ae. aegypti* and other mosquito vectors are available, with initial studies demonstrating their ease and effectiveness in comparison with older methods [66,67]. In Brazil, routine sampling of *Ae. aegypti* adults with gravid traps deployed at relatively low densities was used to identify high risk localities which were then targeted for vector control [68,69]. This 'Intelligent Dengue Monitoring' system was reported to have prevented over 27,000 dengue cases over two 'dengue seasons' between 2009 and 2011 with considerable reductions in cost burden to the communities where it was deployed [70].

None of the studies reported on viral infection rates in the vector. This perhaps is not surprising given that techniques suitable for application in routine surveillance, such as PCR or NS1, have not been available until recently, that vector infection rates with dengue virus are of the order of 1% even in areas where transmission is ongoing [64,70–72] and the cost of running the large numbers of tests to detect meaningful infection levels could be considered prohibitive for many authorities. Nonetheless, routine screening for dengue virus of trapped adult female *Aedes aegypti* is possible and has been incorporated into the routine surveillance program in Belo Horizonte, Brazil [73]. The relative low dispersal rates of *Ae. aegypti* as compared with the high mobility of humans as they commute daily from the home to the workplace, school, etc., means that virus-infection rates in the vector potentially could provide an accurate or epidemiologically valid indicator of dengue risk in any particular locality, thus informing vector control. Clearly, elucidating the relative value of such an index would require substantial research investment, while integrating it into routine surveillance programmes would demand significant sustained investment, but the importance of metrics like the sporozoite or entomological inoculation rates used in malaria epidemiology [59] already indicate the potential.

This review has also demonstrated the unreliability of accepted vector thresholds for dengue transmission. A number of studies reported dengue transmission at BI levels below the currently accepted threshold of 5 (Figure 2) [29,34,36–38] or when the HI was below 1% [74,75]. Elsewhere, Focks proposed a pupal productivity index of 0.25 as a threshold for dengue transmission in Honduras [76], yet in Brazil dengue transmission occurred at PPI levels of 0.15 [58]. While the desire for a single globally applicable transmission threshold is understandable, it seems unlikely that such a threshold exists, given the variety and complexity of other parameters that potentially influence the risk of outbreaks today [19,77,78]. Chadee concluded in 2009 that dengue transmission occurs, not at a fixed entomologic figure/quantity but rather at a variable level based on numerous factors including seroprevalence, mosquito density and climate [30]. It is becoming increasingly apparent that thresholds differ at different locations and in different contexts, and while they must be calculated independently at each location [19,79]. Moreover, empirically defining thresholds, which must be expected to be dynamic, rising and falling as the susceptibility of the local population changes, will require comprehensive prospective, longitudinal vector studies [80], with simultaneous monitoring of the relationship between *Ae. aegypti* population densities and dengue virus transmission in a spatially relevant human cohort.

Study Limitations

In spite of reference searches and use of grey literature, publication bias will likely remain given the very nature of a systematic review. However, we also sought to further limit the effect of publication bias by placing no restriction on language, and those languages encountered were: English, French, Portuguese, Spanish and Chinese.

Additionally, one should be cautious when interpreting these data due to the study design of the 18 articles. As defined by QATAS assessment methods, study design was often weak (15 studies), meaning that studies were prone to bias and confounding factors, which may have skewed some of the reported associations. In addition, most ($n = 13$) studies relied on dengue case data from external sources, rather than obtaining study-generated data. With the exception of vector sampling and generation of vector index, there were few similarities in the approaches across the different studies.

Conclusions and Recommendations

Despite the widespread practice of collecting vector population data, the review has revealed that very few rigorous studies have been undertaken to determine the relationship between vector abundance and dengue transmission; of those that have been published, few provide tangible evidence of such a relationship, and therefore it is not possible to draw a firm conclusion. After decades of vector surveillance in many countries and considering the magnitude of the dengue threat today both in those and other countries that have recently experienced major dengue outbreaks, this is disappointing. Yet it is also indicative of the lack of basic knowledge of dengue epidemiology, in particular with regard to transmission. Clearly, this is a major knowledge gap that requires attention with a degree of urgency and the following research priorities are recommended:

- The relationship between vector population abundance and dengue transmission remains unknown and should be quantified. Studies should aim to collect new vector and clinical datasets carefully matched temporally and spatially. Given that epidemiology will vary considerably between different contexts and geographical localities, multiple locations should be investigated.

- The ideal and most powerful approach would be for a series of coordinated studies, to be carried out in multiple locations worldwide, as exemplified by recent examples [80]. To facilitate such studies, and ensure higher power in individual and combined datasets, the development of a standardized study design and protocols is a priority.
- Individual locations are also strongly encouraged to investigate the relationship independently. Many dengue-affected areas (cities, districts or similar spatial units) are likely to have substantial historic vector and dengue data that potentially may be suitable for appropriate analysis.
- Spatial heterogeneity and transmission at sites other than the home must be considered and carefully incorporated into any study design.
- The utilization of single global values of the Breteau (BI) or other vector indices as thresholds for dengue transmission is unreliable and is not recommended.
- While the need for a standardized reliable definition of a dengue outbreak has already been stated elsewhere [81], research into the relationship between vector abundance and dengue transmission should endeavor to develop a similar approach to defining reliable locality-specific vector population indices (*e.g.* thresholds, rates of increase, etc.) for use as early warning signals for impending increases in dengue transmission.
- Adoption of adult dengue vector sampling by all vector surveillance programs is urged. Various new trapping methods, as well as a simple resting catch approach, should be evaluated.
- Relationship between larval, pupal and adult stages of the vector population and the factors influencing adult emergence rates remain poorly understood. The paucity of fundamental knowledge of the ecology of mosquito vectors generally and the need for basic studies has been advocated elsewhere [82,83] and is true for *Ae. aegypti* and *Ae. albopictus*. A greater understanding of the ecology of dengue vectors is essential.

In the absence of definitive evidence that dengue vector surveillance data can contribute to the prediction of dengue outbreaks, it might be tempting to consider abandoning the practice altogether. However, this would be a rash and premature judgment. At the very least, this systematic review has demonstrated that the potential of vector surveillance data has not yet been evaluated. Indeed, its full potential will not be apparent until its contribution to a complete predictive model incorporating all other covariates influencing dengue epidemiology have been considered. That will not be possible until multiple high quality studies investigating the relationship between vector populations and dengue transmission have been carried out.

Supporting Information

Checklist S1 PRISMA checklist.
(DOC)

Table S1 Data extraction table summary for reviewed studies.
(XLSX)

Table S2 Assessment of the validity of reviewed studies: Table of bias and QATQS (Quality Assessment Tool for Quantitative Studies) rating for each study.
(XLSX)

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Author Contributions

Conceived and designed the experiments: LRB PJM. Analyzed the data: LRB. Wrote the paper: PJM LRB SRR. Contributed to the review protocol: LRB SRR PJM. Carried out literature searches: LRB. Reviewed the literature: LRB PJM. Wrote the conclusions and article drafts: PJM LRB. Drafted the manuscript: PJM LRB. Reviewed and approved the final manuscript: LRB SRR PJM.

References

1. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, et al. (2010) Dengue: a continuing global threat. Nature Publishing Group 8: S7–S16. doi:10.1038/nrmicro2460.
2. Simmons CP, Farrar JJ, Nguyen VVC, Wills B (2012) Dengue. N Engl J Med 366: 1423–1432. doi:10.1056/NEJMra1110265.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution and burden of dengue. Nature 496: 504–507. doi:10.1038/nature12060.
4. World Health Organization (2012) Global strategy for dengue prevention and control 2012–2020. World Health Organization. 43 pp. Available: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf
5. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, et al. (2012) Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis 6: e1760. doi:10.1371/journal.pntd.0001760.s009.
6. Luz PM, Vanni T, Medlock J, Paltiel AD, Galvani AP (2011) Dengue vector control strategies in an urban setting: an economic modelling assessment. The Lancet 377: 1673–1680. Available: http://www.sciencedirect.com/science/article/pii/S0140673611602468.
7. Undurraga EA, Halasa YA, Shepard DS (2013) Use of expansion factors to estimate the burden of dengue in southeast Asia: A systematic analysis. PLoS Negl Trop Dis 7: e2056. doi:10.1371/journal.pntd.0002056.s003.
8. Shepard DSD, Undurraga EAE, Halasa YAY (2013) Economic and disease burden of dengue in southeast Asia. PLoS Negl Trop Dis 7: e2055–e2055. doi:10.1371/journal.pntd.0002055.
9. Halasa YA, Shepard DS, Zeng W (2012) Economic cost of dengue in Puerto Rico. Am J Trop Med Hyg 86: 745–752. doi:10.4269/ajtmh.2012.11-0784.
10. Martelli CMT, Nascimento NE, Suaya JA, Siqueira JB, Souza WV, et al. (2011) Quality of life among adults with confirmed dengue in Brazil. Am J Trop Med Hyg 85: 732–738. doi:10.4269/ajtmh.2011.11-0067.
11. Lambrechts L, Scott TW, Gubler DJ (2010) Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. PLoS Negl Trop Dis 4: e646. doi:10.1371/journal.pntd.0000646.t002.
12. Barrera R, Amador M, MacKay AJ (2011) Population Dynamics of Aedes aegypti and Dengue as Influenced by Weather and Human Behavior in San Juan, Puerto Rico. PLoS Negl Trop Dis 5(12): e1378. doi:10.1371/journal.pntd.0001378
13. Campbell KM, Lin CD, Iamsirithaworn S, Scott TW (2013) The Complex Relationship between Weather and Dengue Virus Transmission in Thailand. Am J Trop Med Hyg 89: 1066–1080. doi:10.4269/ajtmh.13-0321.
14. World Health Organization (2009) Dengue: Guidelines for diagnosis, treatment, prevention and control. WHO, Geneva, Switzerland: 1–160. Available: http://www.ncbi.nlm.nih.gov/books/NBK143157
15. Focks D (2004) A review of entomological sampling methods and indicators for dengue vectors. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases: 1–40. Available: http://www.who.int/iris/handle/10665/68575#sthash.OmTDGEBh.dpuf
16. Connor ME, Monroe WM (1923) Stegomyia indices and their value in yellow fever control. Am J Trop Med Hyg 1: 9–19. Available: http://intl.ajtmh.org/content/s1-3/1/9.full.pdf

17. Focks D, Chadee DD (1997) Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. Am J Trop Med Hyg 56: 159–167. Available: http://www.ncbi.nlm.nih.gov/pubmed/9080874
18. Tun-Lin W, Kay BH, Barnes A, Forsyth S (1996) Critical examination of *Aedes aegypti* indices: correlations with abundance. Am J Trop Med Hyg 54: 543–547. Available: http://www.ncbi.nlm.nih.gov/pubmed/8644913
19. Kuno GG (1995) Review of the factors modulating dengue transmission. Epidemiol Rev 17: 321–335. Available: http://www.ncbi.nlm.nih.gov/pubmed/8654514
20. Sanchez L, Cortinas J, Peleaz O, Gutierrez H, Concepción D, et al. (2010) Breteau index threshold levels indicating risk for dengue transmission in areas with low *Aedes* infestation. Tropical Medicine & International Health 15: 173–175. Available: http://www.ncbi.nlm.nih.gov/pubmed/20409286
21. Scott TW, Morrison AC (2003) *Aedes aegypti* density and the risk of dengue virus transmission. In: Takken W, Scott TW (eds) Ecological aspects for application of genetically modified mosquitoes. FRONTIS, Dordrecht, The Netherlands, pp 187–206. Available: http://library.wur.nl/ojs/index.php/frontis/article/view/849
22. Focks D, Brenner RJ, Hayes J, Daniels E (2001) Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am J Trop Med Hyg 62: 11–18. Available: http://www.ajtmh.org/content/62/1/11.full.pdf#page = 1&view = FitH.
23. Ellis AM, Garcia AJ, Focks D, Morrison AC, Scott TW (2011) Parameterization and sensitivity analysis of a complex simulation model for mosquito population dynamics, dengue transmission, and their control. Am J Trop Med Hyg 85: 257–264. doi:10.4269/ajtmh.2011.10-0516.
24. Andraud M, Hens N, Marais C, Beutels P (2012) Dynamic epidemiological models for dengue transmission: A systematic review of structural approaches. PLoS ONE 7(11): e49085. doi:10.1371/journal.pone.0049085
25. Badurdeen S, Valladares DB, Farrar J, Gozzer E, Kroeger A, et al. (2013) Sharing experiences: towards an evidence based model of dengue surveillance and outbreak response in Latin America and Asia. BMC Public Health 13: 1–1. doi:10.1186/1471-2458-13-607.
26. Centre for Reviews and Dissemination (2009) Systematic Reviews: CRD's guidance for undertaking reviews in health care. York: University of York; 2009. Available: http://www.york.ac.uk/inst/crd/pdf/Systematic_Reviews.pdf
27. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. Open Med 3: e123–e130. Available: http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1000097
28. National Collaborating Centre for Methods and Tools (2008) Quality assessment tool for quantitative studies. Hamilton, ON: McMaster University. (Updated 13 April, 2010). Retrieved from http://www.nccmt.ca/registry/view/eng/14.html.
29. Sanchez L, Vanlerberghe V, Alfonso L, Marquetti MDC, Guzman MG, et al. (2006) *Aedes aegypti* larval indices and risk for dengue epidemics. Emerging Infect Dis 12: 800–806. Available: http://wwwnc.cdc.gov/eid/article/12/5/05-0866_article.htm
30. Chadee DD (2009) Dengue cases and *Aedes aegypti* indices in Trinidad, West Indies. Acta Trop 112: 174–180. doi:10.1016/j.actatropica.2009.07.017.
31. Pham HV, Doan HTM, Phan TTT, Minh NNT (2011) Ecological factors associated with dengue fever in a central highlands province, Vietnam. Stoch Environ Res Risk Assess 25: 485–494. Available: http://ovidsp.ovid.com/ovidweb.cgi?T = JS&CSC = Y&NEWS = N&PAGE = fulltext&D = emed10&AN = 2011358286.
32. Gurtler RE, Garelli FM, Coto HD (2009) Effects of a five-year citywide intervention program to control *Aedes aegypti* and prevent dengue outbreaks in northern Argentina. PLoS Negl Trop Dis 3: e427. Available: http://ovidsp.ovid.com/ovidweb.cgi?T = JS&CSC = Y&NEWS = N&PAGE = fulltext&D = emed9&AN = 2009223964.
33. Katyal R, Kumar K, Gil KS, Sharma RS (2003) Impact of intervention measures on DF/DHF cases and *Aedes aegypti* indices in Delhi, India: An update, 2001. Dengue Bulletin 27: 163–167. Available: http://repository.searo.who.int/bitstream/123456789/15910/2/dbv27p163.pdf
34. Chadee DD, Williams FL, Kitron UD (2005) Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998. Tropical Medicine & International Health 10: 748–754. doi:10.1111/j.1365-3156.2005.01449.x.
35. Romero-Vivas CME, Falconar AKI (2005) Investigation of relationships between *Aedes aegyp*ti egg, larvae, pupae, and adult density indices where their main breeding sites were located indoors. J Am Mosq Control Assoc 21: 15–21. Available: http://www.ncbi.nlm.nih.gov/pubmed/15825756
36. Foo LC, Lim TW, Lee HL, Fang R (1985) Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. The Southeast Asian Journal of Tropical Medicine and Public Health 16: 560–568. Available: http://www.ncbi.nlm.nih.gov/pubmed/3835698
37. Sulaiman S, Pawanchee ZA, Arifin Z, Wahab A (1996) Relationship between Breteau and House indices and cases of dengue/dengue hemorrhagic fever in Kuala Lumpur, Malaysia. J Am Mosq Control Assoc 12: 494–496. Available: http://www.ncbi.nlm.nih.gov/pubmed/8887232
38. Honorio NA, Nogueira RMR, Codeco CT, Carvalho MS, Cruz OG, et al. (2009) Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil. PLoS Negl Trop Dis 3(11): e545. doi: 10.1371/journal.pntd.0000545
39. Rubio-Palis Y, Perez-Ybarra LM, Infante-Ruiz M, Comach G, Urdaneta-Marquez L (2011) Influence of climatic variables on dengue cases and abundance of *Aedes aegypti* (Diptera: Culicidae) in Maracay, Venezuela. Boletin De Malariologia Y Salud Ambiental 51: 145–157. Available: http://www.scielo.org.ve/scielo.php?pid = S1690-46482011000200004&script = sci_abstract&tlng = en
40. Lin CH, Wen TH (2011) Using geographically weighted regression (GWR) to explore spatial varying relationships of immature mosquitoes and human densities with the incidence of dengue. IJERPH 8: 2798–2815. Available: http://www.mdpi.com/1660-4601/8/7/2798
41. Chaikoolvatana A, Singhasivanon P, Haddawy P (2007) Utilization of a geographical information system for surveillance of *Aedes aegypti* and dengue haemorrhagic fever in north-eastern Thailand. Dengue Bulletin 31: 75–82. Available: http://repository.searo.who.int/handle/123456789/16080
42. Chadee DD, Shivnauth B, Rawlins SC, Chen AA (2007) Climate, mosquito indices and the epidemiology of dengue fever in Trinidad (2002–2004). Ann Trop Med Parasitol 101: 69–77. doi:10.1179/136485907X157059.
43. Correa PR, Franca E, Bogutchi TF (2005) Aedes aegypti infestation and occurrence of dengue in the city of Belo Horizonte, Brazil. Rev Saude Publica 39: 33–40. Available: http://www.ncbi.nlm.nih.gov/pubmed/15654458
44. Fernández R W, Lannacone O J, Rodríguez P E, Salazar C N, Valderrama R B, et al. (2005) Population behavior of *Aedes aegypti* larvae for estimating dengue fever cases in Yurimaguas, Peru, 2000–2004. Rev Peru Med Exp Salud Publica 22: 175–182. Available: http://www.scielo.org.pe/scielo.php?pid = S1726-46342005000300004&script = sci_abstract&tlng = en
45. Arboleda SS, Jaramillo-O NN, Peterson ATA (2012) Spatial and temporal dynamics of *Aedes aegypti* larval sites in Bello, Colombia. J Vector Ecol 37: 37–48. doi:10.1111/j.1948-7134.2012.00198.x.
46. Hanna JN, Ritchie SA, Phillips DA, Serafin IL, Hills SL, et al. (2001) An epidemic of dengue 3 in far north Queensland, 1997–1999. Medical Journal of Australia 174: 178–182.
47. Macdonald WW (1956) *Aedes aegypti* in Malaya. II. Larval and adult biology. Ann Trop Med Parasitol 50(4): 399–414.
48. Vazquez-Prokopec GM, Kitron U, Montgomery B, Horne P, Ritchie SA (2010) Quantifying the spatial dimension of dengue virus epidemic spread within a tropical urban environment. PLoS Negl Trop Dis 4(12): e920. doi:10.1371/journal.pntd.0000920
49. Yoon I-K, Getis A, Aldstadt J, Rothman AL, Tannitisupawong D, et al. (2012) Fine scale spatiotemporal clustering of dengue virus transmission in children and *Aedes aegypti* in rural Thai villages. PLoS Negl Trop Dis 6(7): e1730. doi:10.1371/journal.pntd.0001730
50. Schafrick NH, Milbrath MO, Berrocal VJ, Wilson ML, Eisenberg JNS (2013) Spatial clustering of *Aedes aegypti* related to breeding container characteristics in coastal Ecuador: Implications for dengue control. Am J Trop Med Hyg 89: 758–765
51. Duncombe J, Clements A, Davis J, Hu W, Weinstein P, et al. (2013) Spatiotemporal patterns of *Aedes aegypti* populations in Cairns, Australia: assessing drivers of dengue transmission. Tropical Medicine and International Health 18: 839–849. doi:10.1111/tmi.12115
52. Kroeger A, Lenhart A, Ochoa M, Villegas E, Levy M, et al. (2006) Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. BMJ 332: 1247–1252
53. Stoddard ST, Forshey BM, Morrison AC, Paz-Soldan VA, Vazquez-Prokopec GM, et al. (2013) House-to-house human movement drives dengue virus transmission. Proc Natl Acad Sci 110(3):994–9. doi: 10.1073/pnas.1213349110
54. Stoddard ST, Morrison AC, Vazquez-Prokopec GM, Paz Soldan V, Kochel TJ, et al. (2009) The role of human movement in the transmission of vector-borne pathogens. PLoS Negl Trop Dis 3(7): e481. doi:10.1371/journal.pntd.0000481
55. Morrison AC, Sihuincha M, Stancil JD, Zamora E, Astete H, et al. (2006) *Aedes aegypti* (Diptera: Culicidae) production from non-residential sites in the Amazonian city of Iquitos, Peru. Ann Trop Med Parasitol 100 Suppl 1:S73–S86. doi:10.1179/136485906X105534
56. World Health Organization (2000) Strengthening implementation of the global strategy for dengue fever/dengue haemorrhagic fever prevention and control. Report of the Informal Consultation 18–20 October 1999, Geneva WHO/CDS/(DEN)/IC/2000.1 Available: http://www.who.int/csr/resources/publications/dengue/whocdsdenic20001.pdf
57. Barrera R, Amador M, Diaz A, Smith J, Munoz-Jordan JL, Rosario Y (2008) Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. Med. Vet. Entomol 22: 62–69. doi: 10.1111/j.1365-2915.2008.00720.x.
58. Pilger D, Lenhart A, Manrique-Saide P, Siqueira JB, Da Rocha WT, et al. (2011) Is routine dengue vector surveillance in central Brazil able to accurately monitor the *Aedes aegypti* population? Results from a pupal productivity survey. Tropical Medicine & International Health 16: 1143–1150. doi:10.1111/j.1365-3156.2011.02818.x.
59. Anders KL, Hay SI (2012) Lessons from malaria control to help meet the rising challenge of dengue. Lancet Infec Dis 12(12) 977–984. doi: 10.1016/S1473-3099(12)70246-3.
60. Goh KT (1997) Dengue–a re-emerging infectious disease in Singapore. Ann Acad Med Singap 26: 664–670.
61. Maciel-de-Freitas R, Peres RC, Alves F, Brandolini MB (2008) Mosquito traps designed to capture *Aedes aegypti* (Diptera: Culicidae) females: preliminary comparison of Adultrap, MosquiTRAP and backpack aspirator efficiency in a dengue-endemic area of Brazil. Mem Inst Oswaldo Cruz 103: 602–5.

62. Barrera R, Mackay AJ, Amador M (2013) An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. Parasit Vectors 6: 225. doi: 10.1186/1756-3305-6-225.
63. Ritchie SA, Buhagiar TS, Townsend M, Hoffmann A, Van Den Hurk AF, McMahon JL, Eiras AE. (2014) Field validation of the gravid *Aedes* trap (GAT) for collection of *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 51: 210–9.
64. Ritchie SA, Pyke AT, Hall-Mendelin S, Day A, Mores CN, et al. (2013) An explosive epidemic of DENV-3 in Cairns, Australia. PLoS ONE 8(7): e68137. doi:10.1371/journal.pone.0068137.
65. Barrera R (2011) Spatial stability of adult *Aedes aegypti* populations. Am J Trop Med Hyg, 85:1087–1092 doi:10.4269/ajtmh.2011.11-0381.
66. Maia MF, Robinson A, John A, Mgando J, Simfukwe E, Moore SJ (2011) Comparison of the CDC Backpack aspirator and the Prokopack aspirator for sampling indoor- and outdoor-resting mosquitoes in southern Tanzania. Parasites & Vectors 4:124. Available: http://www.parasitesandvectors.com/content/4/1/124
67. Vazquez-Prokopec GM, William A. Galvin WA, Kelly R, Kitron U (2009) A new, cost-effective, battery-powered aspirator for adult mosquito collections. J Med Entomol 46: 1256–1259. Available: http://www.ncbi.nlm.nih.gov/pubmed/19960668
68. Eiras AE, Resende MC (2009) Preliminary evaluation of the 'Dengue-MI' technology for Aedes aegypti monitoring and control. Cad Saude Publica 25 Suppl 1:S45–58. doi: 10.1590/S0102-311X2009001300005.
69. Pepin KM, Marques-Toledo C, Scherer L, Morais MM, Ellis B, et al. (2013) Cost-effectiveness of novel System of mosquito surveillance and control, Brazil. Emerging Infect Dis 19(4) 542. doi 10.3201/eid1904.120117.
70. Mammen MP Jr, Pimgate C, Koenraadt CJM, Rothman AL, Aldstadt J, et al. (2008) Spatial and temporal clustering of dengue virus transmission in Thai villages. PLoS Med 5(11): e205. doi:10.1371/journal. pmed.0050205.
71. Garcıa-Rejon J, Lorono-Pino MA, Farfan-Ale JA, Flores-Flores L, Rosado-Paredes E, et al. (2008) Dengue virus-infected Aedes aegypti in the home environment. Am J Trop Med Hyg 79: 940–950. Available: http://www.ajtmh.org/content/79/6/940.long.
72. Yoon I-K, Getis A, Aldstadt J, Rothman AL, Tannitisupawong D, et al. (2012) Fine scale spatiotemporal clustering of dengue virus transmission in children and Aedes aegypti in rural Thai villages. PLoS Negl Trop Dis 6(7): e1730. doi:10.1371/journal.pntd.0001730.
73. de Figueiredo RMP, Gomes Mourão MP, Conte Abi-Abib YE, de Oliveira CM, Roque R, de Azara T, Ohly J, Degener C, Geier M, Eiras AE (2013) Identification of dengue viruses in naturally infected *Aedes aegypti* females captured with BioGents (BG)- Sentinel traps in Manaus, Amazonas, Brazil. Revista da Sociedade Brasileira de Medicina Tropical 46:221–222. http://dx.doi.org/10.1590/0037-8682-1096-2013
74. Goh KT, Ng SK, Chan YC, Lim SJ, Chua EC (1987) Epidemiological aspects of an outbreak of dengue fever/dengue haemorrhagic fever in Singapore. The Southeast Asian Journal of Tropical Medicine and Public Health 18: 295–302. Available: http://www.ncbi.nlm.nih.gov/pubmed/3433161
75. Koh BKW, Ng LC, Kita Y, Tang CS, Ang LW, et al. (2008) The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. Ann Acad Med Singap 37: 538–545. Available: http://www.google.co.uk/search?client = safari&rls = en-us&q = The+2005+dengue+epidemic+in+Singapore+epidemiology+prevention+and+control&ie = UTF-8&oe = UTF-8&redir_esc = &ei = Uj2IT8_UG8G_0QWc1um7CQ.
76. Focks DA, Daniels E, Haile DG & Keesling JE (1995) A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation, and samples of simulation results. American Journal of Tropical Medicine and Hygiene 53: 489–506.
77. Reich NG, Shrestha S, King AA, Rohani P, Lessler J, et al. (2013) Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. Journal of The Royal Society Interface 10: 86. doi:10.3201/eid1302.060539.
78. Rabaa MA, Simmons CP, Fox A, Le MQ, Nguyen TTT, et al. (2013) Dengue virus in sub-tropical northern and central Viet Nam: Population immunity and climate shape patterns of viral invasion and maintenance. PLoS Negl Trop Dis 7(12): e2581. doi:10.1371/journal.pntd.0002581
79. Sommerfeld J, Kroeger A (2012) Eco-bio-social research on dengue in Asia: a multicountry study on ecosystem and community-based approaches for the control of dengue vectors in urban and peri-urban Asia. Pathog Glob Health 106: 428–35. doi: 10.1179/2047773212Y.0000000055.
80. Morrison AC1, Gray K, Getis A, Astete H, Sihuincha M, Focks D, Watts D, Stancil JD, Olson JG, Blair P, Scott TW. (2004) Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. J Med Entomol 41:1123–42.
81. Runge-Ranzinger S, Horstick O, Marx M, Kroeger A (2008) What does dengue disease surveillance contribute to predicting and detecting outbreaks and describing trends? Tropical Medicine & International Health 13: 1022–1041. doi:10.1111/j.1365-3156.2008.02112.x.
82. Ferguson HM, Dornhaus A, Beeche A, Borgemeister C, Gottlieb M, et al. (2010) Ecology: a prerequisite for malaria elimination and eradication. PLoS Med 7:e1000303. doi: 10.1371/journal.pmed.1000303.
83. Godfray HC (2013) Mosquito ecology and control of malaria. J Anim Ecol 82(1): 15–25. doi: 10.1111/1365-2656.12003.